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Physiological Zoölogy

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ANATOMICAL PATTERNS AND BEHAVIOR PATTERNS

C. JUDSON HERRICK
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ANIMAL behavior is behavior of something. Having determined what an animal does, we are interested to learn how he does it and what he does it with.

The simpler patterns of vertebrate behavior are usually described in terms of reflexes and the linkage, recombination, and conditioning of these units. Reflex behavior is commonly regarded as the operation of a more or less stable anatomical arrangement of neurons connected as reflex arcs or circuits. The tendency is to regard these as enduring anatomical patterns which are given in the innate organization and to conceive them as the starting-point of an analysis of the nervous apparatus in all more complex forms of behavior.

But the observed behavior does not always conform with this scheme and the known structure of the nervous system is equally at variance with it. It is the organism as a whole that is behaving, not merely the reflex arcs. Indeed, the nervous arcs habitually employed in the performance of some act may be seriously damaged by the destruction of important links in the chain of conductors and after a time a readjustment is made so that the accustomed behavior is carried on with the aid of apparatus never before used in this way. The ability of the individual to make the appropriate reaction is not dependent upon the integrity of those particular parts of the nervous system hitherto employed.

There is of late a strong trend in general biology, in neurophysiology, in psychology, and in philosophy toward a search for mech-

anisms of integration. We are no longer content with analysis of the body by anatomical methods and the investigation of the functions of the several parts. The attempt now so popular to limit the scope of physiology to this procedure and to claim for psychology the whole field of integrated activity and adjustment of the organism as a whole is repudiated by every general physiologist and by most biologists. Nor are we content with verbal formulations of theoretic unifying principles.

We want to see the physical machinery of integration in action and to have an intelligible account of it. The rigidity of the reflex arc concept disqualifies it as an adequate statement. In biology we envisage the organism as a whole, set in a particular environment to which it reacts in a fashion which is something more than the mathematical sum or resultant of the interplay of its reflexes. Patterns of behavior emerge which cannot be reduced to mere concatenations of rigid reflex units. The organization is not merely constructive; it is creative, and until we have brought to light the apparatus of this creative power the scientific problem is unresolved.

The Gestalt movement in psychology is perhaps the most significant of the protests against the inadequacy of traditional mechanistic explanations of behavior. But this and most of the other organismic formulations leave the biologist somewhat confused, for he has difficulty in picturing a mechanism which can deliver so intangible and incomprehensible a product as a "configuration on a ground." He demands the exhibition of some sort of bodily organs with assignable functions before taking these intangibles very seriously.

This demand has been fairly well satisfied for plants and very lowly animals by Child's doctrine of physiological gradients and centers of dominance, and by the discovery of various chemical and other integrating agencies. But the behavior of vertebrates presents much more exacting demands which have not been fully met. Possibly if we go back as far as we can toward origins of vertebrate behavior and the apparatus employed, the problem may be set in clearer light.

There are two groups of simply organized vertebrates that present the physiological problem reduced almost to lowest terms. These are the cyclostome fishes (lampreys and hagfish) and the

urodele amphibians (larvae of all forms and the "permanent larvae" like *Necturus*). The nervous systems of these animals have been explored to learn just what is the anatomical mechanism of their simple behavior patterns.

The brains of hagfishes (*Bdellostoma* and *Myxine*) have been exhaustively studied by Dr. J. L. Conel (development) and Dr. Jan Jansen (adult structure) and their reports will shortly appear in the *Journal of Comparative Neurology*. The organization of these most primitive of all vertebrates has been further simplified by a semi-parasitic habit. Two senses—smell and touch—are very highly developed and the other special senses are in various stages of degeneration. There are vestigial eyes, and no jaws or limbs, and the visible behavior is reduced to a very simple system of primitive body movements.

The brain is organized around the two dominant sensory systems, the olfactory nerve and the trigeminal nerve, and the simple motor mechanism is activated chiefly from these two sources of excitation. One would perhaps hope to find so elementary a brain easy to analyze anatomically. The simple and very stereotyped action-system might be expected to require only a few well-defined cerebral centers cross-connected by a small number of clearly separated and well-insulated correlation tracts of nerve fibers. But this is very far from what is actually found.

These little brains are, in fact, the most difficult to analyze microscopically that we know in the whole range of vertebrate neurology. The nerve centers, even the largest of them, have vague outlines and show very little internal specialization. They are cross-connected by innumerable very fine and much branched fibers which are rarely gathered up into well-defined bundles or tracts. The correlating fibers form a dense web of inextricably entangled axons that seem at first view to run everywhere without plan or meaning. The axon of a single neuron may divide repeatedly and the branches may go to widely separated and apparently unrelated parts of the brain; it is hard to see how any specific function can be localized anywhere. The whole fabric seems to be almost equipotential with very little possibility of confining any nervous impulse to any particular reflex circuit.

This seems like a very anomalous and inexplicable arrangement. Here is an adult animal with the simplest and most rigidly stereotyped action-system known among the vertebrates, with few reflexes of very simple pattern, and these carried out with great precision and regularity; and yet with a brain showing less clearly defined reflex centers and less segregation of the correlating fibers into separate tracts than any other. The brain seems to be nearly equipotential in all parts except the primary sensory and motor centers directly connected with the peripheral end-organs, and yet this brain is the adjusting mechanism of a very rigid system of simple reflexes. How does it do it?

Turning now to the amphibian nervous system, we meet much the same situation, especially in the tadpoles and the adults of the more primitive species. But in both hagfish and salamanders the first impression of meaningless confusion and lack of localizable functional specificity yields to a more detailed examination of the actual structure.

The application of refined neurological technique to these refractory tissues and patient unraveling of the texture by following out the full courses of individual nerve fibers in all their ramifications and the determination of all of the connections of particular neurons with location of the synaptic junctions—this sort of examination shows that the organization of these primitive brains is not disorderly, though the structural plan of the organization is very different from that of a properly wired house or factory, in which every electric circuit is perfectly insulated from every other one.

It is to be borne in mind that the normal action system of these primitive vertebrates is chiefly total reactions involving in each case almost the entire body musculature. The typical response to almost any kind of adequate stimulus is a wriggle. The body musculature and the peripheral neurons directly connected with it are very perfectly arranged to execute this wriggle promptly and vigorously. The sense organs are well developed and highly specialized—a few in the hagfish and more in the salamander—but in response to the stimulation of these sense organs singly or in combination there follows (outside the head) always one kind of movement, a wriggle. There are no fingers and toes, no hands and feet, to complicate the behavior pattern.

The development of this wriggly behavior in salamanders has been minutely described by Coghill. He has followed its growth stage by stage from the first futile twitch up to perfectly co-ordinated swimming; and he has recounted the steps in the maturing of the nervous mechanism employed, showing exactly what new details of nervous connections are added with each increment in the complexity of the behavior pattern.

From these studies it is very clear that the swimming rhythm has not been built up by assembling the separate movements as preformed simple reflexes, but that each stage has grown out of the preceding and simpler stage, and that each stage in the development of the reaction is a physiological unit completely co-ordinated as far as it goes. This co-ordination is not something added to bind the separate reflexes together and integrate them into a complex swimming movement. It is present from the start; indeed, it is present before any movement of body muscles is possible, for the preceding stages of growth and differentiation have been ordered and integrated by the same fundamental physiological processes that form the organic background and matrix within which the reflexes are matured and linked in effective patterns.

The growing egg of the salamander is an integrated body from the start and the first nervous mechanisms of adjustment and integration emerge within this orderly dynamic pattern and are ordered by it. And indeed throughout life the matured nervous functions continue to work, not in isolation and autonomously, but always in relation with the non-nervous activities of the rest of the body and of the external environment, by which in fact their patterns are in large measure controlled.

These generalizations are not armchair speculations. They are well founded upon an enormous mass of careful observational and experimental work. In preneural stages we have the physiological gradients of Child, chemical regulators of various sorts, and doubtless many other integrating agencies less fully known. The multiplication and differentiation of the nervous elements can be seen to go on in an orderly way within a dynamic field with unity and a pattern of organization determined by the organismic processes just mentioned.

The first reflex response to external stimulation arose not as an isolated event, but within this dynamogenic field and as an integral part of it. These early reflexes, in turn, play their own part in shaping the further course of growth. Intrinsic and extrinsic agencies cooperate from the beginning of development in shaping the course of growth and differentiation (Child) and they continue to do so throughout the maturing and conditioning of the reflexes. Many of these agencies have been brought to light and all are seen to fit into a single dynamic pattern—the organism as a whole—which is the resultant of the interplay of all of them.

Now, as already indicated, Coghill has described the sequence of anatomical changes in the reacting mechanism that parallels the observed changes in the behavior pattern. When new reflexes appear, or new patterns of linkage of simple reflexes, i.e., when new things are learned, the new circuit is not carved out of the undifferentiated matrix as an independent structural unit; but in general the new arc is formed by extension or expansion of the total pattern with individuation within it and modification of the already existing and functioning circuits. The vital unity of organization is preserved unbroken with every extension of its range and efficiency. This is the organic background within which every new configuration of structure and behavior is set and out of which it has emerged.

The more primitive types of reflex as seen in young amphibian larvae and adult hagfish are mass movements of the whole body musculature. All sensory excitations converge ultimately into the common final paths leading to this musculature. Axons from the primary olfactory, tactile, and other sensory centers pass directly to the motor centers in more or less well-defined tracts. From these axons numerous collateral branches are given off, and these may spread widely to almost all other parts of the brain.

At first these collateral connections are few in number and the behavior patterns are correspondingly few and inflexible. But not wholly so, for even at this early stage various non-nervous influences may alter the course of the reflexes served by these simple arcs; they work in a total organic situation and are influenced by it.

As collateral connections between the reflex arcs increase in number and in range of complexity, there is provided the machinery for

reinforcement, or for inhibition of tactile excitations by olfactory or of olfactory by tactile, and so on. There is no cerebral cortex or any other highly elaborated mechanism of correlation and integration. It is done by the reciprocal interaction of each sensory system with all of the others through the diffuse nervous meshwork or neuropil whose anatomical analysis has been so difficult.

This is the integrating machinery, and it is adequate for the purpose so long as the chief organs of response are not very complicated—a single system of body muscles always acting, if at all, in much the same way. The "ground" on which a particular "configuration" of behavior is set has an anatomical organ within the brain, the relatively equipotential neuropil which pervades the brain substance and binds it all together as a functional unit. Into this neuropil there is a continuous discharge from every sensory surface of the body, fluctuating in character from moment to moment, but always acting. Here growth is going on as long as learning is possible, here the total pattern expands, and here partial patterns are individuated. This neuropil when more highly elaborated is the machinery of conditioning of reflexes as Pavlov describes it.

The dynamics of the pattern of this groundwork of the individual is in part repetitive response to stimulation in stereotyped molds, typified by reflex and habit; and in part it is change in the organization of the material—growth. All learning is growth. It involves a change, more or less stable, in the anatomical structure of the body, and the neuropil as the most labile part of the nervous system plays the most significant part in this reorganization of the mechanisms of behavior.

The behavior pattern exhibits a certain permanence and stability because the larger features of bodily organization are permanent. The organs of response are limited in number—in the lowly animals here under consideration—and they are limited in the range of movements permitted by their structure; all parts of the central nervous adjustor may and ordinarily do discharge their activities by few final common paths into this limited repertoire of muscular organs. There is also a large measure of fluidity, of impressionability to transient experiences, because the dynamic pattern is the interplay of nervous excitations from all parts of the body within the nervous adjustor,

and this adjustor is labile and readily modified under the influence of the personal experiences of the individual. During growth "excitation from the environment, internal and external, simply stamps specific behavior value upon, or into, this growing pattern."[1]

Now a particular sense organ is stimulated, say by odorous emanations in the water. The pathway of maximal nervous discharge from the primary olfactory center is by way of the direct tract of nerve fibers down to the motor centers. But the collateral discharge into the general field of neuropil is also very extensive, and this neuropil also discharges downward into these same motor centers. Just what the animal does in response to the stimulation will depend on the resultant of the action of the direct path and of the indirect way through the diffuse neuropil. If the animal is hungry the neuropil, influenced by the general condition of the body, will reinforce the olfactory excitation and a seeking reaction results. If the animal is already satiated, the influence from the neuropil will be an inhibition and no reaction follows.

Here we have recognizable anatomical organs for a particular "configuration" of behavior—a phasic reaction to a stimulus—and also for the organic "ground" within which that reaction is set and of which it is an expression. The account, of course, is schematic and incomplete, but as far as it goes it is a fairly accurate glimpse of the actual organization of a hagfish or a young salamander larva. Yet even here there is the beginning of the differentiation of a more efficient sort of nervous adjustor which takes the form of localized regions, or centers, in each of which some particular system of correlations is facilitated. And from this humble beginning the cerebral cortex arises.

With elaboration of more complicated organs or response—jaw muscles and eye muscles and hands and feet—the primitive apparatus of integration and adjustment of behavior just described is obviously inadequate. Within the one dominant system of final common paths we now have many subsidiary systems, and each of these requires its own central adjustor; above these there must be higher centers of correlation which control them all and prevent them from

[1] Quoted from Coghill in a personal communication; compare his *Anatomy and the Problem of Behavior*, p. 97.

working at cross-purposes. Thus the unity of the organization and its behavior are preserved throughout the process of differentiation.

These correlation centers emerge from the relatively equipotential neuropil at the chief crossroads of traffic, at strategic points for the integration of particular types of behavior—reticular formation, cerebellum, midbrain, thalamus, corpus striatum, cerebral cortex. When this process of differentiation is well advanced the brain exhibits two sharply contrasted sorts of structural arrangements. One of these is a complicated system of clearly defined and physiologically specific local centers connected by compact strands of well-insulated nerve fibers. These serve the elementary reflexes and other stable or stereotyped behavior patterns. The other is neuropil in the correlation centers which retains some of the non-specific and plastic character of the primordial neuropil of the ancestral vertebrates.

The non-specific or plastic nervous tissue is not diminished in higher brains with their elaborate provision of localized centers and tracts. It is increased, for the gray matter of the higher correlation centers—particularly that of the thalamus and cortex of cerebellum and cerebrum—continues to provide the general organic groundwork of the personality as well as to add to this the apparatus of conditioning of reflexes, of learning, of memory, and of all the intelligent adjustments.

The human organism is still a biological unit, with all of the nervous and non-nervous machinery of integration possessed by our lowly ancestors and much more besides, which they lack. We have added the apparatus of conscious experience and of evaluation of that experience in relation to the self and the social milieu, and this too is set in the same organic groundwork as are our reflexes and instincts. There is a physical apparatus for every "configuration" of conscious experience, and the "ground" in which this "configuration" is set is the sum-total of the personality with a physical apparatus which is the whole organization in relation to its environment. The dynamic patterns of the "ground" act largely at subconscious levels, so that their effects on the conscious experience cannot always be discovered by introspection.

The full significance of a human personality can be appreciated only by looking at it from as many different angles as possible. In

particular, the introspective experience must be supplemented by biological examination of its genetic and phylogenetic origins and by investigation of that structural organization which has the experience. In this last inquiry our interest is not confined to the study of the organs of consciousness, the machinery of emotion and intellect and volition; but it extends beyond this to the whole apparatus of our unconscious physiological life, for all of this is part of the groundwork of the conscious experience.

The psychological unity of the individual personality and of the separate acts which he performs is not a disembodied perfecting principle or any other abstraction. It is the integrated operation of a physical body so organized that its nervous and non-nervous activities work together harmoniously to give orderly behavior somewhat as on a simpler scale the parts of a loom co-operate to deliver a particular pattern of woven fabric.

Neither Gestalt nor any other psychological principle or technique, like psychoanalysis, can attain full scientific completeness until we have found out how it articulates with the vital process as a whole; and this means, among other things, that we must find a physical organization adequate for the task. We already know where to look for this, but full knowledge of just how it works is likely to be long delayed.

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THE DIFFERENTIAL REDUCTION OF OSMIC ACID IN THE CORTEX OF PARAMECIUM, AND ITS BEARING UPON THE METABOLIC GRADIENT CONCEPTION[1]

(One plate and twelve text figures)

ORLANDO PARK
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USING the Kolatschev-Nassonov osmication technique, as described in a previous article (Park, 1929), I have been able to confirm certain observations made by Nassonov (1924), who impregnated the excretory apparatus of a number of protozoans with osmic acid. The field of inquiry has been extended by the discovery of a gradient of osmiophilic substances in the ectoplasm of *Paramecium caudatum*. This gradient exists in the form of blackened granules which are more conspicuous in the posterior region, and decrease in intensity toward the anterior region. This differential in reduction of osmic acid has also been found in the excretory apparatus, in which the posterior contractile vacuolar system is in most cases more heavily impregnated than the anterior contractile vacuolar system.

MATERIALS AND METHODS

Paramecium caudatum Ehr. was obtained in abundance from mixed and pure cultures in decaying vegetation, as well as from infusions of hay and of wheat grains. A species of *Vorticella* was obtained from an old, clear infusion of boiled hay.

The technique of Nassonov (1924, pp. 442–45) was followed (Park, 1929). Impregnation was from 46 to 120 hours, at a temperature of from 30° to 35°C. Apparently there is for each species an optimum period of impregnation time which must be determined before the best results are obtained. This period may vary also with the race of animals used, their physiological condition, and the strength of the osmic acid. Thus for *Paramecium* of my cultures,

[1] This work was done in 1925, and throughout the writer has had the constant advice of Dr. B. H. Willier. I am also indebted to Dr. C. M. Child and to Dr. R. R. Bensley for timely criticism.

an impregnation of 96 hours at 30°C. usually gave the best results, although in some cultures the impregnation ran over a hundred hours at 36°C. to obtain approximately the same picture. Again, the vacuolar system was apparently best brought out in the fission cycle and poorly in the conjugation cycle, which substantiates, in part, the conclusion reached by Unger (1925). *Vorticella* was fixed for 30 hours and impregnated for 106 hours at 30°C. The material was observed both as whole mounts, and in serial sections cut at six microns.

OBSERVATIONS AND DISCUSSION

1. *The ectoplasmic gradient in osmiophilic substances.*—In the impregnated material of *Paramecium caudatum*, it is to be noted that the posterior end of the animal is usually darker in color, and that this intensity of the blackening reaction tends to fall off gradually toward the anterior end (Plate I, Figs. 1, 2; text Fig. 5). This gradient in the differential reduction of osmic acid by osmiophilic substances exists mainly, if not wholly, in the ectoplasmic layer (Plate I, Figs. 3, 4; text Fig. 6). On the whole, there is less reduction of osmic acid in the entoplasm, and the blackening is of more uniform density throughout. This is to be expected in consequence of the entoplasmic currents. Thus at the anterior end, osmication in the entoplasm more nearly approximates osmication in the ectoplasm, in contrast to the situation at the posterior end of the individual where osmication is much greater in the ectoplasm (Fig. 6).

In the material examined, where the optimum of impregnation has been approximated, this situation is in accord with the conception of the metabolic gradient in infusoria as advanced by Child (1914), and substantiated by Child and Deviney (1926), and Petersen (1927).

Child and Deviney (*loc. cit.*), working with *Paramecium caudatum*, observe (p. 304) that the "reduction gradient with low concentrations of methylene blue and the indophenol reaction suggests ectoplasmic differences in oxidative activity along the axis," and (p. 311) that ". . . . the various data indicate the existence of a physiological gradient in the longitudinal axis. . . . . The evidence points to the conclusion that the gradient is primarily a quantitative differential including both metabolic and physical factors."

Such a conception has received further support by Petersen (*loc. cit.*) using the same species of ciliate. She found that (p. 158) in "the division of the first filial generation at 26° to 30°C., the anterior cell always divided before the posterior; at 13 to 17 degrees, the posterior piece always divided first; and at 18 to 22 degrees the relative rate varied," and further, that these results indicate that "the sudden rise of temperature accelerates, and the lowering of temperature retards physiological activity to a greater extent in the anterior than in the posterior region of the body. . . . ."

If such a gradient in oxidative metabolism exists in the ectoplasm of *Paramecium caudatum*, being more pronounced at the anterior end and gradually decreasing in intensity toward the posterior end, a reversed gradient as described above, in the amount or nature of oxidizable materials, or waste products, might be expected to occur.

2. *The contractile vacuolar system in Paramecium caudatum.*—In *Paramecium caudatum* the walls of both vacuole and vacuolar canals are well defined as black lines of varying width after treatment with osmic acid. Both whole mounts and sections gave good results. The sections, especially, showed the vacuolar system largely as depicted by Nassonov (*loc. cit.*) and Gelei (1928).

Only rarely do both vacuoles impregnate equally, the posterior contractile vacuolar system being usually much more heavily blackened and draining a larger area than the anterior contractile vacuolar system (Fig. 7).

It is possible that the differences between the anterior and posterior contractile vacuolar systems in the relative amount of area occupied as suggested above, intensity of blackening with osmic acid, and degree of complication, may be correlated with the contractile vacuolar rate, as various authors have noted that the anterior contractile vacuole has a more rapid pulsation rhythm than the posterior.

If, as has been suggested, the posterior end has a less intense oxidative metabolism, and, as shown in this article, this level of the ectoplasm has a higher capacity for osmication, then the heavier impregnation of the posterior vacuole would be expected, as it lies near the surface, and being subject to the physiological conditions

PLATE I

FIG. 1.—*Paramecium caudatum*. Photomicrograph, ×350. Whole mount. Series PTA-1. Osmic impregnation 46 hours, 35°C.

FIG. 2.—*Paramecium caudatum*. Photomicrograph, ×350. Whole mount. Series I1-B. Osmic impregnation 90 hours, 35°C.

FIG. 3.—*Paramecium caudatum*. Photomicrograph, ×350. Serial, 6 microns. Series PY-2. Osmic impregnation 96 hours, 30°C.

FIG. 4.—*Paramecium caudatum*. Photomicrograph, ×350. Serial, 6 microns. Series PY-3. Osmic impregnation 96 hours, 30°C.

FIG. 13.—*Paramecium caudatum*. Photomicrograph, ×1,000. Whole mount. Contractile vacuole in optical section, showing phase between diastole and systole, with distended ampullae. Note the osmiophilic walls and blackened feeding canal.

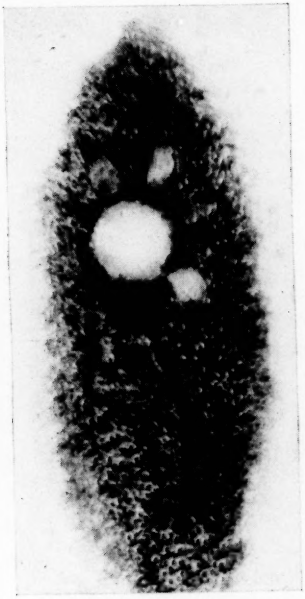
PLATE I

1

2

3

4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of this level, its rate tends to become slower. Whether this lower rate of the posterior vacuole is due to a retarding effect of osmiophilic substances, or other oxidizable materials, or to some other differences in physiological condition between anterior and posterior regions, is not certainly known. It is possible that the lower rate is due to a deficiency in oxidase activity between anterior and posterior regions. It has been shown by Child and Deviney (1926) that the indophenol reaction occurs more strongly in the anterior region, which would

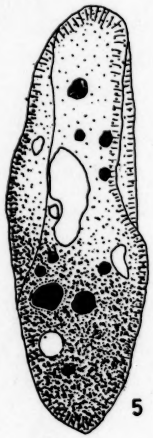

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FIG. 5.—*Paramecium caudatum*. Whole mount, Series F-3, ×900. Osmication gradient.

FIG. 6.—*Paramecium caudatum*. Serial, 6 microns, Series Q-2, ×900. Anteroposterior gradient in osmiophilic substances; note light, uniform osmication of entoplasm.

indicate such a condition. Any generalization here, however, is open to doubt. Further work with osmic-acid impregnation on species of protozoa which have an elongated contractile vacuolar system, as in *Spirostomum*, should yield some data on this and related questions, particularly if correlated with physiological observations.

The vacuolar canals are seen as tubes with walls of varying thickness (Figs. 8 and 9). They are well shown near the height of systole, when an ampulla connects the interior of the vacuole with the rest of the feeding canal, but are to be made out in the diastolic

phase as well. These canals generally decrease in diameter as they lead away from the vacuole, finally appearing as fine, black lines, or they may end abruptly in the cytoplasm (Fig. 9). They are seldom

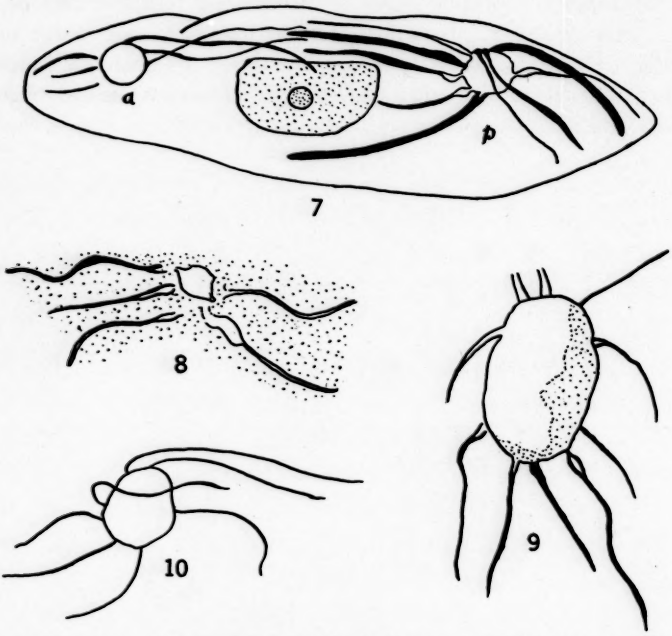

FIG. 7.—*Paramecium caudatum*. Series PTa-6, ×900. Anterior (*a*) and posterior (*p*) contractile vacuoles with their systems of feeding canals. Note the length of the canals, one from each vacuole reaching the meganucleus; and the more heavily impregnated condition of the posterior vacuole.

FIG. 8.—*Paramecium caudatum*. Drawn from photomicrograph, ×1,000. Optical section of a contractile vacuole and five feeding canals.

FIG. 9.—*Paramecium caudatum*. Series Q4-12, ×1,000. Contractile vacuole and feeding canals.

FIG. 10.—*Paramecium caudatum*. Series Fa, ×1,000. Contractile vacuole and feeding canals.

straight (in part owing to the shrinking action of osmic acid); instead they tend to curve from the basal end so that they form a spiral around the vacuole (Fig. 10). From one to thirteen canals have been counted, the number usually being from seven to nine.

The canals, as brought out by osmication, are seen to be much longer than supposed. Often a single canal may run nearly two-thirds of the way around the animal. Further, in a cross-section through the vacuole one may find two canals, one leading away from either side of the reservoir, and almost girdling the animal (Fig. 11). Canals may be seen leaving each contractile vacuole and running a more or less tortuous course to a level corresponding with the center of the meganucleus (Fig. 7). In view of the length and number of such canals, it is possible that canals from the two vacuoles may, at some time, become united into a continuous tube, although I have seen no actual indication of such a coalescence in the material studied.

The vacuolar canals traverse a protoplasmic region just beneath what apparently is the trichocyst layer. This protoplasmic layer is an osmiophilic zone of varying thickness and intensity (Fig. 12).

Khainsky (1910), working with *Paramecium caudatum*, found the contractile vacuoles to be formed from accessory vacuoles, and Taylor (1923), using *Euplotes*, explains the mechanism of the contractile vacuole as a sol-gel reversibility process. As Taylor describes it, the vacuole arises from smaller vacuoles, and these from still smaller vacuoles until finally a set of minute vesicles are observed to be at the base of the process. The latter, Taylor thought, arose *de novo* from the cytoplasm. This temporary nature of the contractile vacuole is refuted by Young (1924) who, also using *Paramecium caudatum*, finds no evidence of accessory vacuoles. Others have thought such accessory vacuoles to be of a pathologic nature.

In the *Paramecium caudatum* studied, I have been unable to find any evidence of normal accessory vacuoles, and believe, with Young, that the contractile vacuolar system is a continuous and permanent one. Surely in the animals prepared, some would have been killed at a time when the coalescence of accessory vacuoles was in progress, had they been involved. In properly impregnated animals, the vacuolar system is nearly always defined in black outlines, occasionally in diagrammatic form (Plate I, Fig. 13; text Figs. 16, 17). Often this impregnation of the so-called excretory apparatus is exceedingly heavy, and appears to ramify throughout the ecto-

plasm; in other cases the system is ill defined, irregular, and more or less broken (Fig. 14).

I have found another localization of osmiophilic material. In some individuals, which will be considered at another time, there

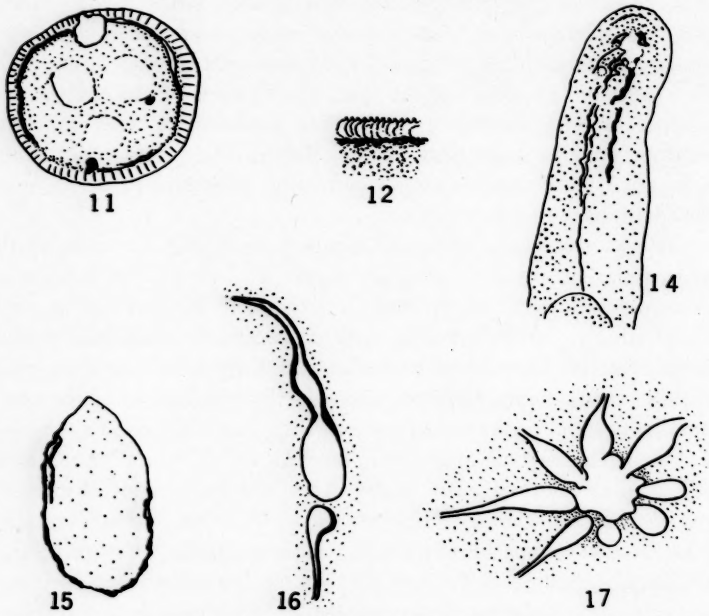

FIG. 11.—*Paramecium caudatum*. Drawn from photomicrograph, ×1,000. Note vacuolar discharge pore and canals, with distinct lumen, almost encircling the animal.

FIG. 12.—*Paramecium caudatum*. Series Q-2, ×1,000. Osmiophilic zone between the surface and the entoplasm.

FIG. 14.—*Paramecium caudatum*. Series Q-2, ×1,000. Anterior contractile vacuole with a feeding canal reaching the anterior margin of the meganucleus.

FIG. 15.—*Paramecium caudatum*. Series Q-2, ×1,000. Meganucleus with osmiophilic material applied to a portion of its surface.

FIG. 16.—*Paramecium caudatum*. Drawn from photomicrograph, ×1,000. Section through a vacuole and two feeding canals with their ampullae.

FIG. 17.—*Paramecium caudatum*. Series PTa-6, ×1,000. Optical section of a contractile vacuole and ampullae.

appears to be an osmiophilic layer more or less closely applied to the meganucleus (Fig. 15). As to the significance of this observation,

little may be said, save to point to the present controversy on the osmiophilic bodies (Golgi bodies?) in protozoa touched upon previously (Park, 1929).

3. *The contractile vacuole in Vorticella.*—The excretory apparatus, so-called, in *Vorticella* was simpler and was observed as a rounded vesicle or vacuole near the surface, with an osmiophilic wall of varying thickness. On the average, this wall took a heavier impregnation than did the contractile vacuole of *Paramecium.* The vacuole has been previously demonstrated by Nassonov (*loc. cit.*) in peritrich infusoria, using the osmic-acid method, and by Fauré-Fremiet (1925). Fauré-Fremiet, also using the modified Kolatschev method, found a permanent, osmiophilic (supposedly lipoidal) wall around the contractile vacuole of *Campanella* and *Epistylis.* In *Campanella umbellaria* he further found two ampulloid canals in communication with the cytopharynx. In the material studied, I was not able to demonstrate as clearly a vacuolar canal system in *Vorticella* as in *Paramecium.* What may prove to be canals were seen at times as short, truncated, blackened lines. These structures may have been due to other factors, as no lumen could be made out. On the other hand, it is possible that a more accurate determination of the range of impregnation might bring out a system of vacuolar canals in *Vorticella.*

4. *Significance of the blackening reaction.*—Our present state of knowledge on osmication precludes any very definite statement of the exact chemical nature of the complex and imperfectly understood blackening reaction. Even if the chemical mechanism is still obscure, the fact remains that there is a definite and easily demonstrated differential reduction of osmic acid in the ectoplasm of *Paramecium caudatum* which is apparently correlated with other previously noted physiological differences along the anteroposterior axis.

SUMMARY

Using an osmic-acid technique, the contractile vacuolar system of *Paramecium caudatum* and of a species of *Vorticella* was brought out in much the same way as that previously described by Nassonov.

In *Paramecium caudatum*, osmiophilic substances were more evident in the fission cycle than in the conjugation cycle. The

osmiophilic material is apparently more abundant in the ectoplasm, where it occupies an osmiophilic zone lying beneath the trichocyst layer. At times what appeared to be an osmiophilic layer was seen investing the meganucleus.

In *Paramecium caudatum* an ectoplasmic gradient in osmiophilic substances was demonstrated, the amount of these substances decreasing from the posterior toward the anterior end. In this species, the posterior contractile vacuolar system was generally more osmiophilic than the anterior.

These observations indicate a possible correlation between the ectoplasmic gradient, its organic composition, the pulsation rate of the contractile vacuole, and the relative size of the area occupied by the latter; further, this evidence appears to be consistent with the conception of the metabolic gradient in infusoria, as advanced by Child.

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FACTORS INFLUENCING THE RATE OF RESPIRATORY METABOLISM OF A DEVELOPING EGG (ORTHOPTERA)

(Fourteen figures)

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INTRODUCTION

DESPITE the fact of their widespread occurrence and extensive use as biological experimental materials, little if any information of a quantitative nature seems to exist concerning the factors involved in the embryological development of insects. Reference to the literature on the question of fundamental changes in single developing insect eggs shows that practically nothing quantitative concerning these interesting phenomena is recorded. Such knowledge as does exist has usually been obtained from experiments carried out on large numbers of eggs and without regard to environmental or other history (Von Fürth, 1903; Krogh, 1916; Winterstein, 1924; Sayle, 1928; and others). In a previous article some facts concerning the effects of temperature on the embryonic development of grasshoppers were presented (Bodine, 1925). The present article is a continuation of this study in which the rates of respiratory exchange and other related phenomena during the development of the eggs have been followed under normal as well as experimental conditions.

It is the specific purpose of these investigations to make as complete a correlation as is possible between the morphology and physiology during the development of these forms so that a more or less rigid standard can be established as a basis for extensive experimental cellular investigations already under way in this laboratory. The material seems of such a unique nature that it will doubtless not be out of place here to point out again some of its outstanding qualifications as experimental biological material. In the first place, the ease with which the eggs can be collected and kept under laboratory conditions is perhaps one of their outstanding qualities. They

can be got in extremely large numbers by collecting females late in the fall and allowing them to deposit their eggs in cages in moist sand. The sand can be sifted at intervals and the exact age of the eggs determined. Since each egg pod contains large numbers of eggs it is an extremely easy task to collect thousands in a relatively short period of time. Eggs can be kept in moist sand out-of-doors during the entire winter. When active developing eggs are desired it is only necessary to put them at a higher constant temperature, e.g., 25° C., and in a very short time rapid development takes place as noted in experiments described here.

Inasmuch as the organism is that of a relatively simple type of cold-blooded animal complicating factors, often encountered in higher forms, are quite lacking or absent. Extensive morphological and cytological investigations already carried out in this laboratory on the group give one an intimate knowledge of cellular conditions not usually available for other forms. The size of the eggs (see Bodine, 1925) is such that it has been found quite possible to dissect out and isolate embryos from the yolk, etc., in very early stages. Cytological technic is greatly simplified by such procedures, and problems as those dealing with cell division, cell growth, differentiation, etc., can be easily worked upon. An attempt is being made to standardize the material so that definite quantitative information can be had concerning the exact morphological and physiological pictures under any known set of environmental conditions of temperature, etc. More exact quantitative correlations will thus be possible for this material than are thus far available for the development of any other form.

MATERIAL AND METHODS

Eggs of ten different species of grasshoppers were used, *Melanoplus differentialis*, *Melanoplus femur-rubrum*, *Circotettix verruculatus*, *Dichromorpha viridis*, *Dissosteria carolina*, *Hesperotettix brevipennis*, *Hesperotettix osceola*, *Romalea microptera*, *Chortophaga viridifasciata*, *Chortophaga australior*. Methods of obtaining, handling, and general procedure for eggs were the same as described previously (Bodine, 1925).

Gas exchange of the developing eggs was measured by both colorimetric and manometric methods previously pointed out

(Bodine and Orr, 1925). The greater part of the observations, however, were made by means of the modified Krogh manometer. In carrying out determinations of gaseous exchange, eggs were used both singly and in lots of ten. Results obtained for lots of ten eggs checked quantitatively with similar observations on single eggs and in many cases such a procedure was adopted so that readings could be made over shorter periods of time than was possible when single eggs were used. Determinations on any one sample of eggs by use of the manometer were never carried out for periods of more than twenty-four hours. Readings were taken at frequent intervals and average rates of gas exchange calculated. Such a method is of importance where possible changes in rates of gas exchange over short periods occur. Constant temperatures were maintained by means of Freas water baths. Weighings of eggs were made on delicate analytical balances.

Water content of eggs was obtained by drying eggs to constant weight in oven at 97° C.

Results reported in this article are based upon experiments carried out over a period of six consecutive years using different eggs for each season so that all possible variations in eggs for different seasons could be satisfactorily checked.

In the present article the physiological side of development in the grasshopper egg will be largely dealt with and reference to morphological findings will only be referred to as matters of especial interest where such seem necessary. In subsequent articles by other workers the morphological studies will be reported in detail. The question of energy of development will also be dealt with at a future time.

NORMAL CONDITIONS FOR DEVELOPING EGGS

In a previous article (Bodine, 1925) it was pointed out that the eggs of grasshoppers can be conveniently grouped into three classes depending upon the temperatures during which embryonic development normally takes place. In Class I, eggs are normally subjected during the winter to low temperatures but will hatch if kept at constant high temperatures from the time of laying. Eggs of this class are normally laid in the late summer and hatch in the early spring. The best example of the class is *Melanoplus differentialis*. In Class

II the eggs are never subjected to low temperatures and do not ordinarily hatch if they are thus treated. Such eggs are laid in late spring and hatch in late summer. *Chortophaga viridifasciata* is perhaps the best example of such a class. In Class III the eggs seem to hatch only if subjected to low temperatures and will not hatch if kept continuously at a constant high temperature. *Circotettix verruculatus*, as pointed out by Carothers (1923), is a classical example of such a group. The present article deals almost entirely with results obtained for eggs of *Melanoplus differentialis* or those represented by Class I. Reference, however, will be made to results obtained for other eggs when comparisons seem necessary in elucidating certain points in the reactions of the Class I eggs. *M. differentialis* in the vicinity of Philadelphia normally lays its eggs out-of-doors during the months of September and early October. The eggs thus deposited in the soil are exposed to the lower temperatures of winter and hatch in the early spring, May and June (Bodine, 1925).

RATES OF OXYGEN CONSUMPTION FOR EGGS KEPT OUT-OF-DOORS

Curve B in Figure 1 shows graphically the rates of oxygen consumption for eggs kept at normal out-of-door temperatures during their entire course of development. This is a composite curve made up of averages taken from experiments at out-of-door temperatures covering a period of some six years. It is of interest to note from this curve that during the period immediately following laying the rates of oxygen consumption rise at a fairly rapid rate and then in November and December, upon the approach of lower temperatures, rapidly fall and remain low over a considerable period, December to February. There then follows a gradual rise in the rate of oxygen consumption which in the early part of April again reaches a more rapid rate that continues to increase up to the time of hatching. As previously pointed out (Bodine, 1925), it would seem that a definite cycle or rhythm in development takes place. The organism first goes through a rapid development and reaches a particular stage in which it undergoes hibernation or exposure to low temperatures. As a matter of fact, as will be shown here, such rhythms seem necessary for the normal development of the organism. Indications of rhythms in the embryonic development of Orthoptera were pointed out by

Wheeler in 1893. A fact of considerable interest, however, is that changes in the rates of oxygen consumption at out-of-door temperatures take place from February to April. During this period the out-of-door temperatures are considerably below the so-called "developmental zero" of the species (Bodine, 1925). It would thus seem that in the case of the grasshopper egg temperature is not necessarily

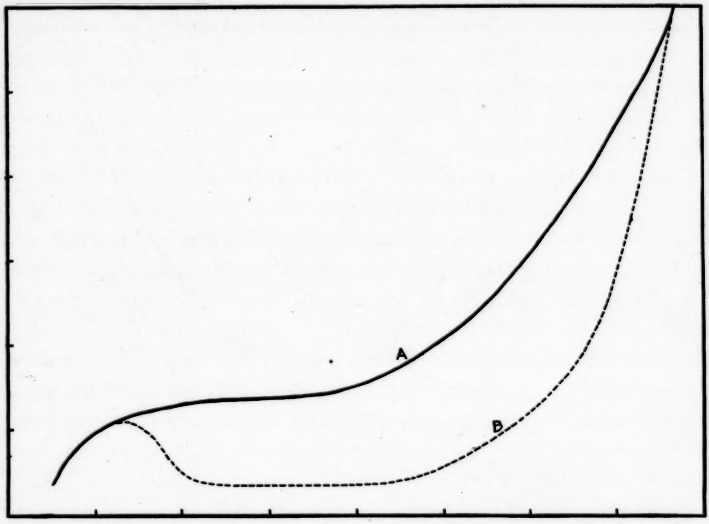

FIG. 1.—Curve A shows rate of oxygen consumption for out-of-door eggs of *M. differentialis* determined at different periods of year at 25°C. Curve B shows rate of oxygen consumption for out-of-door eggs of *M. differentialis* determined at different periods of year at out-of-door temperatures, 0°–20°C. Abscissas, time in months from laying of eggs to hatching. Ordinates, millimeters change of manometer fluid per minute per egg. Actual amounts of oxygen consumed are obtained in all cases by multiplying figure on ordinate by constant factor for manometer. In all experiments curves are for determinations made with same manometer throughout. For further explanation see text.

the limiting factor in controlling the gas exchange during growth or normal development and, as will be shown subsequently, the morphological evidence also indicates that definite development occurs during these periods even though the eggs are exposed to temperatures, supposedly below those required for normal development.

Curve A in Figure 1 and Curves A and B in Figure 2 show the rates of oxygen consumption for out-of-door eggs determined at a constant temperature of 25° and 27° C. These eggs were kept at the constant temperature only long enough for determinations of their oxygen consumption (never more than twenty-four hours) and then subsequently returned to out-of-door conditions. Many similar experiments have been carried out during successive years always with

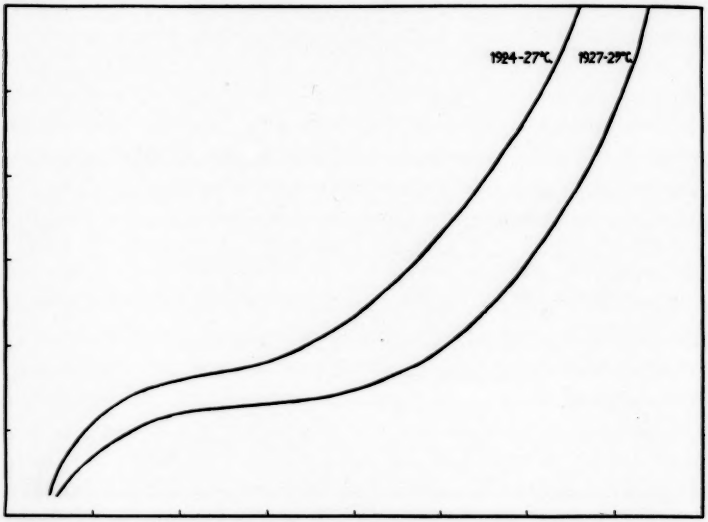

FIG. 2.—Curves show rates of oxygen consumption for out-of-door eggs of *M. differentialis* determined at different periods of the year at 27° and 25°C. Abscissas, time in months from laying of eggs to hatching. Ordinates, millimeters change of manometer fluid per minute per egg. Actual amounts of oxygen consumed are obtained in all cases by multiplying figure on ordinate by constant factor for manometer. Same manometer used throughout for both series of experiments. For further explanation see text.

the same general results. It is of interest to note that in these curves there is also indicated a cycle or rhythm in rates of oxygen consumption during the course of the normal development of the egg, strikingly similar to that indicated in the foregoing for out-of-door eggs at out-of-door temperatures. During the early stages of development the rates of oxygen consumption rise as rapidly as for the eggs

normally out-of-doors. This increase is followed by a much slower one which extends over a considerable part of the winter months similar to the period found for the eggs whose rates of oxygen consumption are determined at normal out-of-door temperatures. In the late winter and early spring marked increases in the rate of oxygen consumption take place up to the time of hatching. This type of curve is always obtained when out-of-door eggs are tested for rates of oxygen consumption at high constant temperatures (23°, 25° and 27° C.). It is, also, of importance to note the increased rates of oxygen consumption of eggs when determined at constant high temperatures over those obtained for similar eggs at out-of-door temperatures. This point will be more fully discussed here in dealing with the effects of temperature on rates of oxygen consumption for eggs under different experimental conditions.

Rates of oxygen consumption for eggs kept only at constant high temperatures from time of laying until hatched.—Since eggs of *M. differentialis* will hatch either at constant high temperatures or when exposed to normal temperature changes of the out-of-doors, it was deemed quite desirable to compare the rates of gaseous exchange of comparable samples of eggs kept under such conditions. Figure 3 shows graphically results of typical experiments for different years for eggs kept only at constant temperatures. An examination of this figure will show that even at constant high temperatures the rates of oxygen consumption show cyclic or rhythmic changes during the course of embryonic development. In other words, differences in rate of gas exchange occur during development at constant high temperatures. A primary rapid increase in rate of oxygen consumption followed by a correspondingly marked decrease always occurs. These in turn are followed by a period of low rates and eventually by a marked increase in rates up to the time of hatching. The extent of these changes in rates is of course conditioned to a great degree by the temperature at which the eggs are kept.

A comparison of these curves with those of Figures 1 and 2 shows the cycles of all to be fundamentally of the same general type. Temperature obviously is not the primary factor in conditioning the cycles since they always occur at constant high temperatures as well as at lower temperatures. It would, therefore, seem that such phe-

nomena are perhaps conditioned by some protoplasmic mechanisms more or less specific (as will be shown later), for the particular organism in question. A point of especial interest is the length of time necessary for development at constant high temperatures as

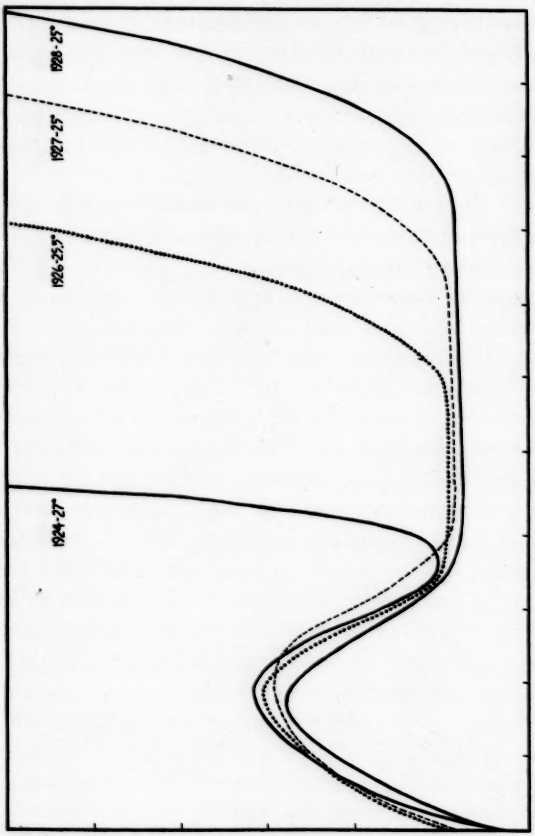

FIG. 3.—Curves show rates of oxygen consumption for eggs of *M. differentialis* kept at constant temperatures from time of laying to hatching. Four separate experiments, carried out during different years, are indicated. Abscissas, time in days. Ordinates, millimeters change of manometer fluid per minute per egg. Actual amounts of oxygen consumed are obtained in all cases by multiplying figure on ordinate by constant factor for manometer. Same manometer used throughout any one series. Experiments for different years not necessarily carried out with same manometers. For further explanation see text.

compared with the much longer time for normal out-of-door conditions. From the standpoint of the dynamics of development such phenomena offer unique opportunities for the accurate estimations of energy requirements under such unusual conditions of cyclic or

rhythmic growth. In a subsequent article this question will be considered in some detail.

Rates of oxygen consumption of normal eggs brought indoors at different periods of the year and kept at constant high temperatures until hatched.—Eggs of *M. differentialis* will hatch when exposed either to constant high temperatures or to an alteration of temperatures as occurs in nature. It, therefore, becomes of interest to determine the effects of putting eggs, normally out-of-doors, at constant high temperatures at different periods during the winter or periods of low temperatures and noting the effects on their rates of oxygen consumption and hatching time. Figure 4 shows graphically results typical of those repeatedly obtained, over a period of some six consecutive years, for eggs brought in from out-of-doors and kept at constant high temperatures until hatched. An inspection of this figure shows several interesting and important facts. Eggs brought indoors and put at constant high temperatures during the initial increase in rate of oxygen consumption, occurring in October and early November, always give rate curves as previously indicated in Figure 3.

After this initial high rate of oxygen consumption is reached out-of-doors and eggs are then subjected to a constant high temperature (25° C.), the types of curves, A, B, C, D, etc., as shown in Figure 4 are always obtained. A short latent period followed by an extremely rapid rate of oxygen consumption occurs up to the time of hatching. It is of interest to note that eggs brought in during the year from out-of-doors have progressively higher initial rates of oxygen consumption and also reach higher final rates at the time of hatching. This point will be subsequently discussed in treating of the energy relations of growth. The first indications of the biological importance of the cyclic or rhythmic rates in oxygen consumption and development seem to appear here. Eggs during their initial high rates of oxygen consumption are not susceptible to, or do not respond when transferred to, constant high temperatures but when thus treated go through the cycle as shown previously in Figure 3. After the peak or optimum initial high rate is reached out-of-doors it is then possible greatly to increase the rates of oxygen consumption as well as the rates of development of the eggs by subjecting them to constant high

temperature (see previous article, Bodine, 1925). It would thus seem that at certain definite stages in the development of the organism at out-of-door temperatures, increased rates at higher temperatures re-

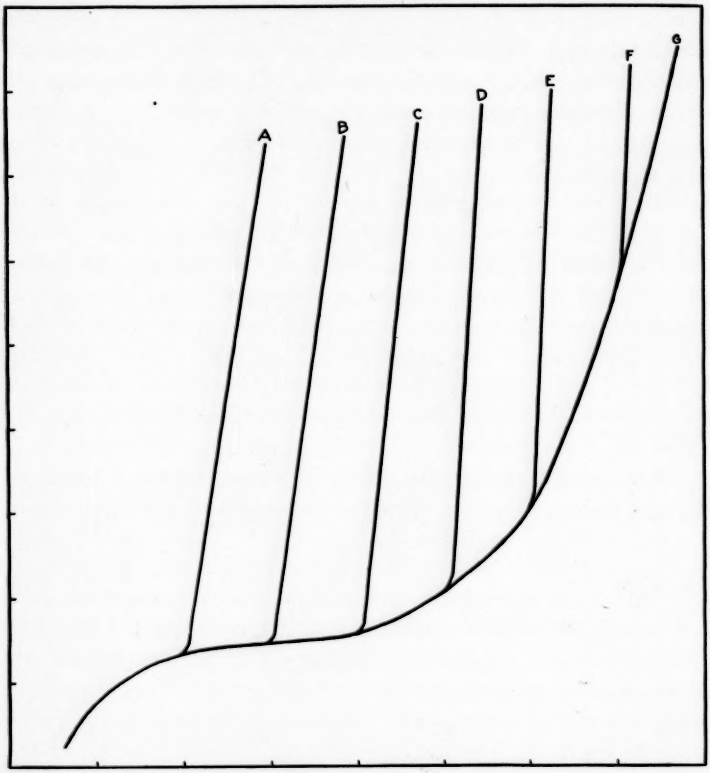

FIG. 4.—Curves show rates of oxygen consumption for out-of-door eggs of *M. differentialis* brought indoors at different periods of the year and kept at a constant temperature (25°C.) till hatched. A, B, C, D, E, F, for eggs brought indoors and kept at 25°C. till hatched. G shows initial rates of oxygen consumption for out-of-door eggs throughout year when put at 25°C. Abscissas, time in months. Ordinates, millimeters change of manometer fluid per minute per egg. Actual amounts of oxygen consumed are obtained in all cases by multiplying figure on ordinate by constant factor for manometer. Same manometer used throughout all experiments. For further explanation see text.

sult. This point has been verified by morphological studies and will be presented in subsequent articles from this laboratory.

That marked physiological as well as morphological changes take place in eggs during their normal exposure to out-of-door temperatures seems clearly demonstrated by the fact that their response during the course of the year when put and kept at constant temperature changes progressively. The same general type of curve for the rates of oxygen consumption is always obtained.

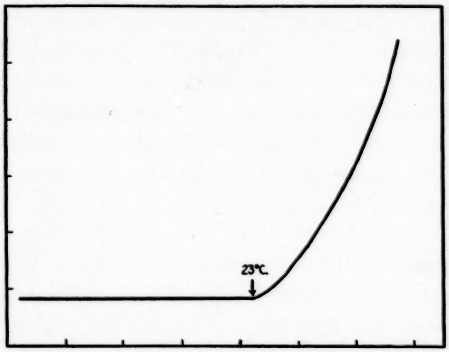

FIG. 5.—Curve shows rate of oxygen consumption at 23°C. for eggs of *M. differentialis* kept at 10°C. from time of laying. Arrow indicates time at which eggs were transferred from 10°C. to 23°C. and left there. Abscissas, time in days. Ordinates, millimeters change of manometer fluid per minute per egg. Actual amounts of oxygen consumed are obtained in all cases by multiplying figure on ordinate by constant factor for manometer. Same manometer used throughout experiment. For further explanation see text.

Since the foregoing results indicate a definite rhythm or cycle of susceptibility during the course of development of the egg it was deemed advisable to determine experimentally if such results could be produced and further information obtained as to the exact periods in development at which such relationships become effective.

Freshly laid eggs were put at a constant temperature of 10° C. during the period of initial increase in rates of oxygen consumption and left there for varying periods of time up to three to four months. Rates of oxygen consumption were determined daily at 23° C., and a typical series of results is represented graphically in Figure 5.

From an inspection of this figure it will be noted that no change in the rate of oxygen consumption occurs in these eggs so long as they are kept constantly at 10° C. After constant exposure to 10° C. for a period of three months the eggs, when then exposed to constant high temperatures, rapidly develop and show a marked increase in the rate of oxygen consumption up to hatching as indicated in Figure 5. The type of this curve is similar to that found for out-of-door eggs when exposed later in the year to constant high temperatures as shown in Figure 4. From these experiments it is difficult to say whether internal changes in the morphology of the embryo are taking place but in a later report this will be more fully discussed.

Experiments have been repeatedly carried out, the results of which indicate quite clearly that eggs pass through definite periods of varying susceptibility to low temperatures. Out-of-door eggs were put at constant temperatures (25° C.) for varying periods and then returned to out-of-door temperatures for intervals of time. They were subsequently put back at 25° C. and kept there until hatched. Rates of gaseous exchange of such eggs were carefully studied. Figure 6 shows graphically results typical of such experiments. From an examination of this figure it is evident that eggs, after alternate periods at 25° C. and out-of-door temperatures, followed by exposure to 25° C., show slight effects of the low temperatures since their rates of oxygen consumption are usually as high as the control eggs kept constantly at 25° C.

A point of especial interest in these experiments, however, is that once the out-of-door egg is stimulated by its initial exposure to 25° C., when subsequently returned to out-of-door low temperatures, no marked decrease in rate of development ensues despite the fact of the exposure to the extremely low temperatures. Development, once started, therefore, seems independent or at least not correlated with external temperatures. There appear, however, to be definite limits within which such reactions work since eggs do not hatch at the lower temperatures if left continuously exposed to them. Reference to Figure 6 shows that out-of-door eggs, kept at 25° C. for different periods and consequently in different stages of development, when exposed to low temperatures show progressively less and less difference in oxygen consumption rates over control eggs. It would thus seem that some mechanism is acting within the egg which

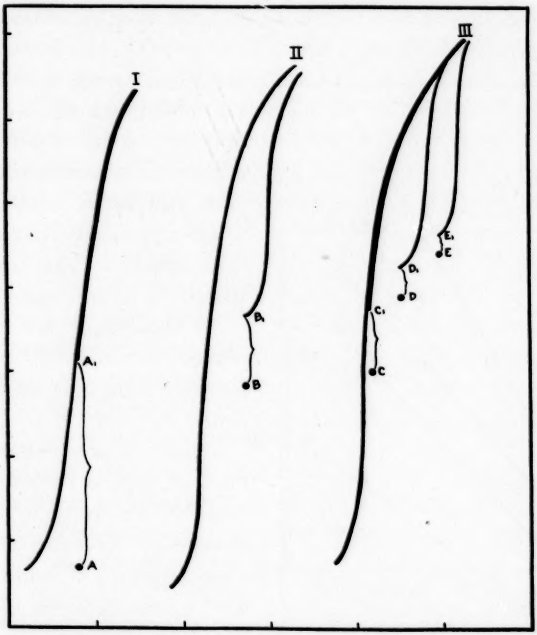

FIG. 6.—Curves showing rates of oxygen consumption for out-of-door eggs of *M. differentialis* when put and left at constant temperature of 25°C., as well as for out-of-door eggs kept at 25°C. for varying periods and then put out-of-doors to be subsequently returned and kept at 25°C. till hatched. All eggs were from the same culture. Abscissas, time. Ordinates, millimeters change of manometer fluid per minute per egg. Actual amounts of oxygen consumed are obtained in all cases by multiplying figure on ordinate by constant factor for manometer. Same manometer used for all eggs. Curve I shows rate of oxygen consumption for out-of-door eggs put and kept at 25°C. till hatched. One-half of culture A was allowed to remain at 25°C. for 2 days, after which it was returned to out-of-doors (0–7°C.) and left there for 6 days; it was then put at 25°C. and left till hatched. Point *A* designates rate of oxygen consumption at end of 2 days at 25°C. Point A^1 designates rate of oxygen consumption when returned to 25°C. after exposure to out-of-doors for six days. Distance AA^1 indicates increase in rate of oxygen consumption over that expected had no development occurred during out-of-door exposure. Curve II, same as I. Culture B at 25°C., 4 days; returned to out-of-doors and left there for 6 days; then put at 25°C. and left till hatched. Points *B* and B^1 as for previous curve. Curve III, same as I. Culture C, 2 days at 25°C., 3 days out-of-doors, CC^1 as in I and II. Culture D, 5 days at 25°C., 4 days out-of-doors, DD^1 as in I and II. Culture E, 7 days at 25°C., 6 days out-of-doors, EE^1 as in I and II. Note decrease in length of *CC*, DD^1, EE^1, indicating shorter and shorter periods during which development continues at out-of-doors as embryo increases in age. For further explanation see text.

makes development in a measure independent of external temperatures; beyond this definite limit, however, the egg becomes quite dependent upon its external environment and its further development seems controlled by temperatures above the "developmental zero" since the egg will not normally hatch at temperatures lower than this value. Results of morphological studies upon these eggs are to be reported later and this point will be more fully discussed at that time.

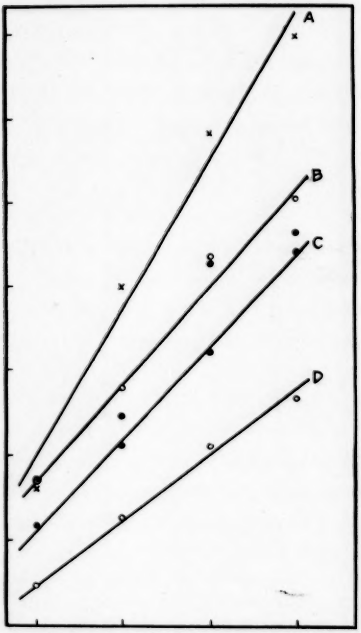

FIG. 7.—Curves showing effect of temperature on rates of oxygen consumption of eggs of *M. differentialis* in different stages of embryonic development. A, for out-of-door eggs after 13 days exposure to 25°C. (ready to hatch); B, for out-of-door eggs after 7–12 days exposure to 25°C.; C, for out-of-door eggs after 5 days exposure to 25°C.; D, for out-of-door eggs without exposure to 25°C. all eggs from same original culture. Abscissas, temperature, degrees centigrade. Ordinates, millimeters change of manometer fluid per minute per egg. Actual amounts of oxygen consumed are obtained in all cases by multiplying figure on ordinate by constant factor for manometer. Same manometer used throughout. For further explanation see text.

Effect of temperature on the rates of oxygen consumption of eggs.—Since rhythmic or cyclic changes in the rates of oxygen consumption are found for eggs kept under different conditions of temperature, etc., it becomes of much interest to determine as far as possible the exact response of eggs of various ages and different developmental histories to temperature. Experiments have been conducted in which out-of-door eggs have been subjected to series of temperatures during the entire course of their development after the initial rise in their rates of oxygen consumption as pointed out above (Fig. 4). Figure 7 shows graphically results of a typical experiment.

From an examination of this figure it is evident that eggs first brought indoors are less responsive to different temperatures than those remaining for a period of a few days at 25° C. As the egg develops its response to temperature becomes progressively greater. That this increased response is not necessarily due to the increase in weight of the embryo can be definitely shown by morphological comparisons of embryo size at these different periods. It is of further interest to note that the quality of the response to temperature at different ages is similar. Somewhat similar results have recently been reported by Melvin (1928) for effects of temperature on lots of eggs of different species although comparisons are scarcely possible because of the wide differences in the types of material worked upon.

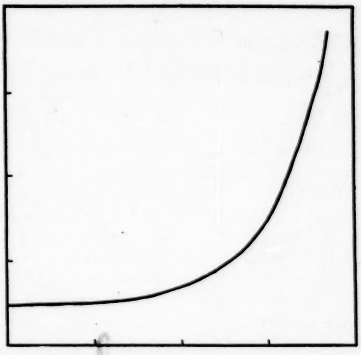

FIG. 8.—Curve showing rate of carbon dioxide output of eggs of *Chortophaga australior* at constant temperature of 23°C. from laying till hatched. All determinations on single eggs. Abscissas, time in days. Ordinates, milligrams carbon dioxide per gram egg per minute. For further explanation see text.

Rates of oxygen consumption of eggs of Class II.—Since eggs of *M. differentialis* are representative of the first class of eggs, pointed out in the preceding, it was thought highly desirable to compare their respiratory metabolism with that of eggs of Class II or those never subjected to low temperatures but developing only at relatively constant high temperatures. Eggs of three such species, *Romalea microptera*, *Chortophaga viridifasciata*, and *C. australior* have been extensively investigated. These eggs were collected in the laboratory at the time of laying and were then put at constant high temperatures as indicated in Figures 8 and 9. Rates of oxygen consumption and carbon-dioxide production of individual eggs were followed during their entire course of development. Figures 8 and 9 show results typical of those obtained for many similar experiments. An inspection of these figures shows that this type of curve is quite different from that shown in the foregoing for *M. differentialis*, in

that no rhythmic or cyclic changes are indicated. A relatively low initial rate of gas exchange over a long period and this followed by a fairly rapid increase up to hatching always occurs. This type of curve is that usually reported for developing insect eggs as indicated by the work of Fink (1925), Melvin (1928), etc. When such eggs are subjected to lower temperatures their rates of gas exchange and hatching time are markedly reduced (Bodine, 1925).

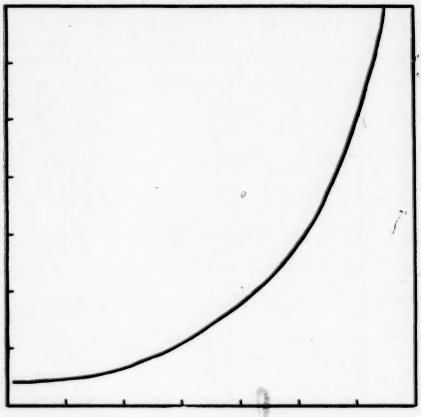

FIG. 9.—Curve showing rate of oxygen consumption of eggs of *Romalea microptera* at constant temperature of 25°C. from laying till hatched. All determinations on single eggs. Abscissas, time in days. Ordinates, millimeters change of manometer fluid per egg per minute. Actual amounts of oxygen consumed are obtained in all cases by multiplying figure on ordinate by constant factor for manometer. Same manometer used throughout. For further explanation see text.

It would thus seem that eggs of Class I as represented by *M. differentialis* go through a cyclic or rhythmic course of development while those of Class II, as pointed out in the preceding, show no such variations.

Respiratory quotients.—Attempts have been made to learn something concerning the types of chemical and other changes taking place during the development of eggs of *M. differentialis*. Since respiratory quotients are used as indices of the types of foodstuffs being consumed (Richardson, 1929) these have been carefully studied. A detailed discussion of the results will be reserved for a subsequent article but it is of considerable interest to note at this time that in all cases throughout the egg's development the values of the respiratory quotient lie between 0.7 and 0.8. No marked changes or deviations in this value have thus far been encountered. It seems reasonable to assume from this value that fats are largely consumed during the development of the organism. A detailed quantitative

microchemical study of this question has been made by Miss E. Slifer of this laboratory and will be reported in the near future.

Changes in water relations of eggs during course of development.—Since eggs of *M. differentialis* were found to be resistant to low temperatures and also readily adaptable to changes in temperature it was thought desirable to investigate rather intensively some physico-chemical properties of the egg which might possibly offer explanations for these phenomena. Extensive studies have accordingly been made on the water relations of the eggs during their de-

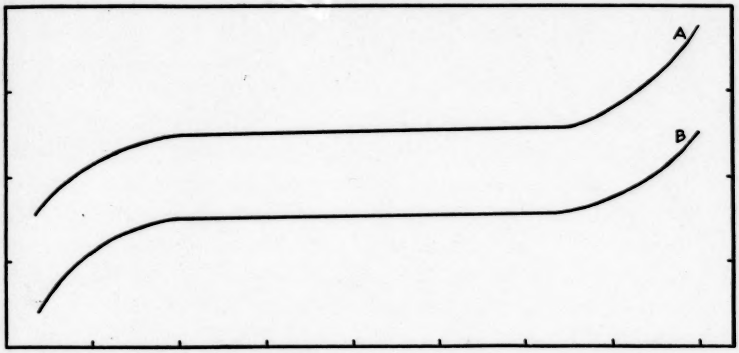

FIG. 10.—Curves showing weight (A), and percentage of water (B) for eggs of *M. differentialis* during entire course of development out-of-doors. Abscissas, time in months. Ordinates, at left, percentage of water; at right, weight per egg, milligrams. For further explanation see text.

velopmental periods under normal as well as experimental environmental conditions. These studies have been carried out over a period of six years and results shown represent mean or average conditions found during this course of time. Many thousands of eggs have been utilized and results checked and rechecked during this period.

Changes in water and weight during normal out-of-door conditions.—Figure 10, in which are shown graphically weight and water changes of the eggs during their normal out-of-door development, is of considerable interest since it too shows cyclic or rhythmic changes similar to those pointed out for the oxygen consumption curves in the foregoing. During the early developmental period of the egg a marked rise in weight and water content results. That this increase in

weight is due solely to water is shown by the fact that the dry weight of the egg remains practically constant until very late in its developmental history. After this initial increase in weight and water and with the approach of cold weather and lower temperatures, the water content of the egg no longer increases but remains practically constant through the winter months or periods of slow development. In the late winter and early spring the eggs again gain weight and increase in water progressively until hatching occurs. These cyclic or rhythmic changes in water with developmental age, as shown in Figure 10, closely resemble the curves in Figure 4, for changes in rates of oxygen consumption, and it is, perhaps, quite reasonable to assume that they are both closely correlated if not expressions of the same things. That changes in the weight of the egg, however, are not the primary causes for the variation in the rates of gaseous exchange of the developing egg becomes evident when actual amounts of gas per unit of embryonic tissue are considered. This point is to be fully discussed in a subsequent report. Changes in water content or changes in the rate of water increases are also probably closely correlated with environmental temperatures of the egg. When low or freezing temperatures occur the egg is prevented from freezing by virtue of its relatively low water content. Such a relation is similar to that found for water relations of a hibernating grasshopper, *C. viridifasciata*, as pointed out previously (Bodine, 1921). Since in *M. differentialis* the egg hibernates, the water regulating or controlling mechanism is relegated to it and the adult animals are unable to withstand low temperatures by virtue of their lack of such a mechanism. In this connection it is of particular interest to note that eggs of Class II, which are not normally exposed to low temperature and in most cases do not hatch if so treated, always have higher water content and do not show such rhythmic or cyclic changes as indicated for eggs of *M. differentialis*.

When eggs of *M. differentialis* are kept at constant high temperatures from time of laying until hatched, cyclic or rhythmic changes in weight and water content are also indicated as shown graphically in Figure 11. The time relations of these changes are much shortened but the type of curve produced is similar to that for out-of-door eggs as shown in Figure 10 above. It is thus quite evident that this water

regulating mechanism is not necessarily conditioned or determined by temperature alone but is more or less specifically bound up with the normal development of the organism.

The water and weight relations as pointed out in the foregoing can and have been experimentally verified repeatedly. In Figure 12 are represented results of a typical experiment showing how the weight and water relations can be experimentally modified. As indicated in this figure, eggs from those kept constantly at high tempera-

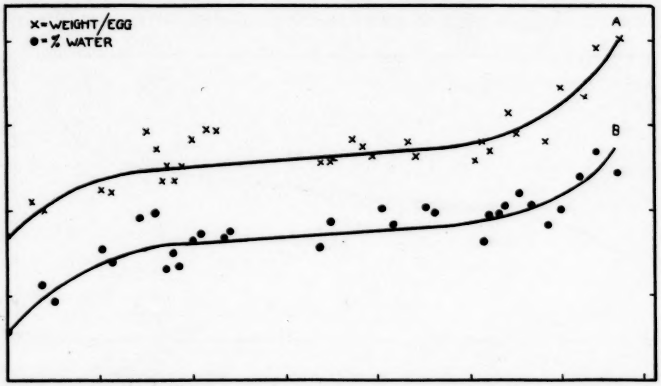

FIG. 11.—Curves showing weight (A) and percentage of water (B) for eggs of *M. differentialis* during entire course of development at 23°C. Abscissas, time in days. Ordinates, at left, percentage of water; at right, weight per egg, milligrams. For further explanation see text.

ture were transferred to out-of-door conditions at varying periods and weight and water changes then followed. Eggs exposed to low temperature after the initial increase in water content remain practically constant during the period of exposure. Eggs placed and kept out-of-doors after the initial rise in water content, however, keep their water relations constant and when again brought indoors and kept at constant high temperature their water content rises rapidly up to the time of hatching. This type of result is invariably obtained when eggs are exposed to low temperatures which are followed by higher constant ones. It thus seems that, experimentally, exact reproductions of normal weight and water relations can be produced.

Hatching time for out-of-door eggs when put and kept at 25° C. at different periods of year.—In a previous article (Bodine, 1925) it was pointed out that eggs of *M. differentialis* normally exposed to out-of-door temperatures when put at a constant high temperature seem to be accelerated in their rates of hatching. Such acceleration was definitely attributed to the action of low temperatures upon the egg. Since these experiments much additional information concerning this point has been accumulated and results are shown graphically in Figure 13. This curve is a composite or average one obtained from thousands of eggs used in gaseous exchange experiments. From an

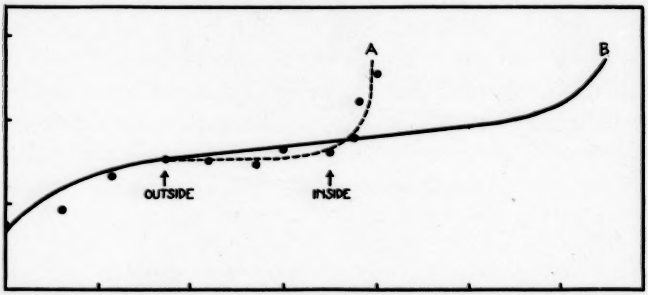

FIG. 12.—Curves showing percentage of water in eggs of *M. differentialis* during entire course of development at 23°C. (B) and also when exposed to initial constant temperature 23°C., to be followed by out-of-doors (0–5°C.) and subsequently returned to 23° till hatched (A). Arrows indicate time eggs were put out-of-doors and subsequently returned inside at 23°C. Abscissas, time in days. Ordinates, percentage of water. For further explanation see text.

examination of this figure it is evident that time for hatching of eggs varies for different periods of the year and that these variations are cyclic or rhythmic, strikingly comparable with similar situations found in gas exchange and weight and water relations of the eggs. Eggs kept at constant high temperatures (25° C.) during their early development in late fall and hence not exposed to low out-of-door temperatures require some hundred days for hatching. With the approach of lower out-of-door temperatures and exposure of the eggs to them, the rates of hatching at constant high temperature are greatly increased. This increase in rate of hatching then decreases gradually as shown in Figure 13, up to the time when the eggs nor-

mally hatch out-of-doors. By comparing this figure with Figure 4 for rates of gas exchange of eggs under identical conditions a marked similarity in shape, etc., will be noted, indicating an obvious correlation between length of incubation period and rates of gas exchange. In a subsequent article dealing with the energy of development this point will be more fully discussed.

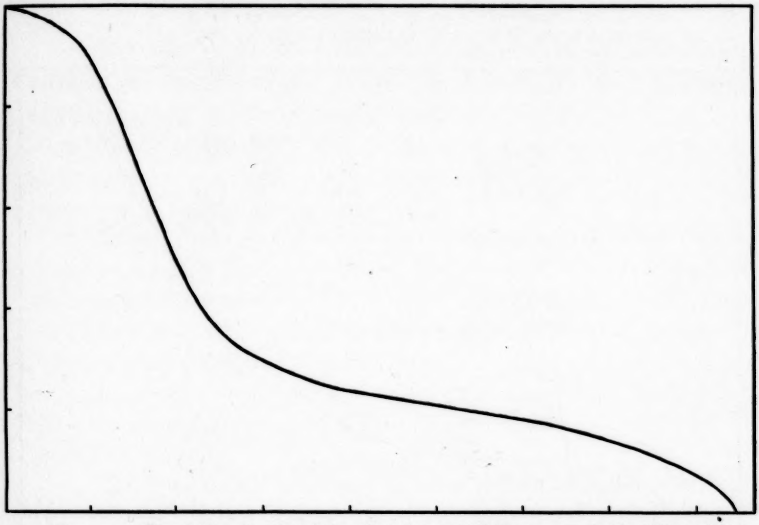

FIG. 13.—Curve showing hatching time for out-of-door eggs of *M. differentialis* when put and kept at 25°C. at different periods of year. Abscissas, time in months. Ordinates, days for hatching at 25°C. For further explanation see text.

DISCUSSION

A detailed discussion of present results and related literature is reserved for a subsequent report dealing with the question of energy of development. In Figure 14, however, there has been brought together a general picture of the changes in the rates of oxygen consumption of developing eggs of *M. differentialis* during exposures to different temperatures. Of particular interest in this figure is the rhythmic or cyclic nature of the gaseous exchange of the eggs irrespective of the temperature at which they are allowed to develop. The marked shortening of the normal out-of-door period is particu-

larly indicated by Curve A for eggs kept only at a constant high temperature (27° C.). The correlations between stage of development and effect of temperature are clearly indicated by Curve B for eggs exposed to constant high temperature after previous exposure to lower out-of-door temperatures. Correlations between physiological and morphological changes in the developing egg are surprisingly clear and definite, and these will be more fully dealt with in subsequent reports from this laboratory.

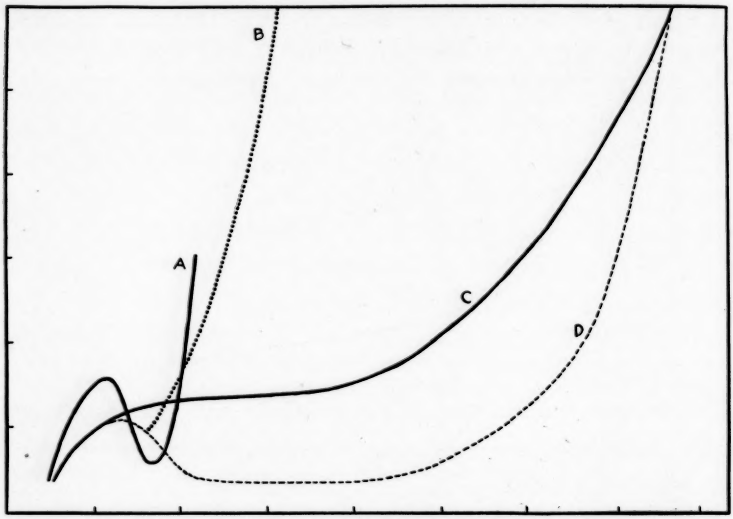

FIG. 14.—Curves showing relative rates of oxygen consumption of eggs of *M. differentialis* when subjected to different environmental temperatures. A, for eggs kept at constant temperature of 27°C. B, for out-of-door eggs put and kept at 25°C. till hatched. C, for out-of-door eggs whose oxygen rates at different times of year were determined at 25°C. D, for out-of-door eggs whose oxygen rates at different times of year were determined at out-of-door temperatures. Abscissas, time in months. Ordinates, millimeters change of manometer fluid per egg per minute. For further explanation see previous figures and text.

A question of considerable biological significance arises after consideration of the foregoing results as to just how far temperature is really a factor in determining the rates of normal development and respiratory metabolism of a cold-blooded animal like an insect and

in how far is one justified in drawing conclusions regarding the effects of temperatures on such development when only time of hatching of the egg is considered as the end-point of the experiment. It seems for the grasshopper egg that differential rates of respiratory metabolism and development are possible at constant temperatures.

SUMMARY AND CONCLUSION

1. Rates of respiratory metabolism during the complete development of the egg of an insect (Orthoptera) under various conditions of temperature, etc., have been presented.

2. Cyclic or rhythmic changes in rates of oxygen consumption and development in eggs, which are apparently independent of environmental temperatures, are pointed out.

3. Differential rates of oxygen consumption and development at constant temperature occur during the development of the egg of *Melanoplus differentialis*.

4. Effects of temperature on the oxygen consumption of eggs of different ages are pointed out.

5. Environmental history is an important factor in determining the rate of oxygen consumption and development in the eggs of certain Orthoptera.

6. The importance of following rates of respiratory metabolism and development from the period of laying to hatching of the egg is indicated.

7. Changes in weight and water relations of developing eggs are given and correlations made between them and the rates of oxygen consumption under normal as well as experimental conditions.

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DARK ADAPTATION IN THE EYE OF ERISTALIS TENAX

(One figure)

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INTRODUCTION

THE process of dark adaptation in photosensory organs has been studied in a few organisms: *Volvox* (Mast, 1911), *Amoeba* (Folger, 1925), three lamellibranchs, *Mya arenaria* (Hecht, 1919 and 1923), *Pholas dactylus* (Hess, 1910, and Hecht, 1927), and *Psammobia vespertina* (Hess, 1910), an ascidian, *Ciona intestinalis* (Hecht, 1918 and 1927), the snail, *Agriolimax campestris* (Crozier and Wolf, 1928), the cephalopod, *Sepia* (Hess, 1910), the molds, *Phycomyces nitens* (Tollenaar and Blaauw, 1921) and *Phycomyces blakesleanus* (Castle, 1929), the amphipod, *Atylus swammerdamii* (Hess, 1910), *Amphioxus* (Hess, 1910), the vertebrates, the chick (Honigmann, 1921), the tadpole (Obreshkove, 1921, and Hecht, 1927) and man (Piper, 1903; Nagel, 1911; and Hecht, 1920, 1921, and 1927). As yet this phenomenon has not been studied in an arthropod, except for the few observations of Hess on *Atylus*. It is important that adaptation be studied intensively in many of the large groups of organisms in the hope of unraveling the processes underlying it and in order to discover if these processes are similar throughout the world of living things.

This article is a first report upon adaptation to light in the eye of the drone fly, *Eristalis tenax*. This organism was chosen because it is an arthropod which can be secured in large numbers and which is especially adapted for experiments of this type owing to its size, hardiness, and uniformly highly positive reactions to light.

METHODS

The insects were collected in the vicinity of the laboratory at Woods Hole, Massachusetts, where they were feeding on various

flowers. They were taken to the laboratory, where after their wings were clipped, they were kept in small wire cages containing cane sugar, privet pollen, and water.

Preliminary experiments showed that specimens with the two eyes unequally adapted to light do not behave in some respects as do animals with both eyes equally light adapted. When a normal specimen is exposed in a field of light formed by two horizontal beams of equal intensity crossing at right angles, it moves in general along the diagonal bisecting the angle formed by the two beams. When, however, a fly with one eye light adapted and the other dark adapted is placed in a similar field it does not move along the diagonal but deflects toward the side of the dark adapted eye, indicating that this eye is more sensitive than the other.[1] The extent of the deflection depends upon the relative sensitivity of the two eyes to light. In the experiments described below advantage was taken of this fact.

The method used in dark adapting one eye and light adapting the other was as follows: A small rectangular box was constructed out of thin sheet copper with the top and one end open. The box was about twice the length of the fly. Its depth and width were such that the insect could be placed in it completely and yet not be crowded. Out of the end of the box and down from the top a slit was cut of a width slightly larger than the width of the neck of the fly. A specimen was placed in the completed box with its body inside, its neck in the slit, and its head outside the end of the box. The organism was held without injury so that it could not move its head. Then over the whole box and insect a piece of tinfoil was wrapped, except that one eye was left exposed and an opening was left at the rear of the insect so that respiration was not interfered with. With a pair of fine forceps the edges of the tinfoil were pressed close around the covered eye. Another piece of tinfoil containing a hole of a diameter about the same as that of the insect's eye was placed around the covered box and fly with the hole in the tinfoil over the uncovered eye. Again the edges of the tinfoil were

[1] This is in harmony with the observation of Garrey (1918, p. 119) that the covering of one eye of the robber fly, *Proctacanthus*, with asphalt varnish causes this eye to become more sensitive to light than the uncovered eye.

pressed tightly around the covered eye. Frequently a third and fourth piece of tinfoil were similarly fastened around the insect. When the operation was complete respiration was not affected and yet one eye was completely covered so that no light could strike it while the other eye was completely uncovered.

Then the insect was placed in an adapting chamber. This was a box (45 by 45 by 270 cm.) made of beaver board and painted dead black inside. Near the center of this box was fastened vertically a 110 v. 150 watt lamp. The insect with only one eye exposed was placed on a horizontal support in the box level with the filament of the lamp. The distance from the filament of the lamp to the eye of the insect was 83.5 cm. The organism faced diagonally toward the light, the long axis being at an angle of 45 degrees with the rays of light. Thus during the exposure to light for the purpose of adaptation the image of the lamp fell on the same region of the retina as it did when the insect was oriented in the field of light consisting of two horizontal beams crossing at right angles.

It is assumed in this article that adaptation to light in one eye does not affect the other. Parsons (1924, p. 54) says, with reference to the human eye, "Dark adaptation of one eye has no effect upon the other."

Before describing these experiments I wish to express my appreciation of the aid of Professor S. O. Mast throughout the course of this work.

RESULTS

The procedure followed and the results obtained may be given by describing the reactions of a typical insect. This organism was collected, brought immediately into the laboratory, and left in a cage before an open window for an hour or more. Then it was taken into the dark room and left in darkness for an hour, after which it was tested in a field of light formed by two horizontal beams crossing at right angles, as described in previous papers (Mast and Dolley, 1924; and Dolley and Wierda, 1929). The luminous intensity of each beam at the center of the field of light was 52.9 m.c. In four trials it made an average deflection of 0.7 degrees toward the side of the left eye from the diagonal bisecting the angle formed by the two beams, its paths thus almost coinciding with the diagonal

(Table I, Exp. I). After this test the insect was placed again in a cage before an open window and allowed to remain there for an hour, i.e., until the eyes became thoroughly light adapted. Then the right eye was covered with tinfoil and the insect was placed in the adaptation chamber as described in the foregoing and left thirty minutes during which the right eye was dark adapted and the left eye light adapted in a luminous intensity of 52.9 m.c. The organism was now taken quickly from the adapting chamber, freed of its wrappings, and removed from the small metal box. The whole operation was done in dim red light and did not take more than 60 seconds. The fly was then tested again in the field consisting of

TABLE I

SENSITIVITY OF EYE OF *Eristalis tenax* TO LIGHT AFTER DARK ADAPTATION FOR 30 MINUTES*

EXPERIMENT	AVERAGE ANGLES OF DEFLECTION IN FOUR TRIALS					TOTAL AVERAGE ANGLE
	Designation of flies					
	a	b	c	d	e	
I	0.7 *l*	9.7 *r*	0.7 *r*	6.2 *r*	16.7 *l*	
II	25.2 *r*	24.0 *r*	17.0 *l*	23.0 *r*	20.2 *r*	
Corrected angle (I+II)	25.9 *r*	14.3 *r*	17.7 *l*	16.8 *r*	36.9 *r*	15.24 *r*

* *r*, deflection toward right; *l*, deflection toward left; Exp. I and II, tests in two horizontal beams crossing at right angles, illumination in each, 52.9 m.c. For procedure preceding each experiment see text.

two horizontal beams precisely the same as in the preceding tests. It now made an average deflection toward the side of the dark adapted eye of 25.2 degrees from the diagonal bisecting the angle formed by the beams (Table I, Exp. II), whereas with the two eyes equally light adapted it deflected 0.7 degrees toward the side of the left eye. Dark adaptation of one eye for 30 minutes consequently resulted in an increase in deflection toward the dark adapted eye of 25.9 degrees. Similar results were obtained in tests on four other specimens. These, together with those referred to in the foregoing, are presented in Table 1.

As is clear from the reactions of the typical insect described in the preceding, each fly before being tested in Exp. I (Table I) had been previously subjected to the following two experimental pro-

cedures: first, both eyes had been adapted to the bright light of the laboratory for 60 minutes or more, and then immediately afterward both eyes had been thoroughly dark adapted for 60 minutes. Immediately after the test in Exp. I and before the test in Exp. II each insect was subjected to the following two experimental procedures: first, both eyes were adapted to the bright light of the laboratory for 60 minutes, and then immediately afterward the right eye was dark adapted and the left eye was light adapted for 30 minutes in an illumination of 52.9 m.c.

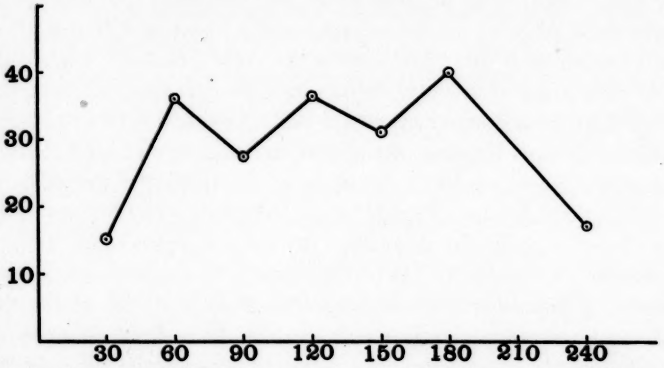

FIG. 1.—Relative sensitivity to light in eye of *Eristalis tenax* after various periods of dark adaptation. Ordinate, degree of sensitivity as indicated by the degree of deflection from the diagonal bisecting the angle formed by two beams of equal illumination, 52.9 m.c., crossing at right angles. Abscissa, duration of dark adaptation in minutes.

As is seen in Table I the five insects with one eye dark adapted and the other light adapted for 30 minutes made an average deflection from the diagonal bisecting the two beams of 15.24 degrees toward the side of the dark adapted eye.

In the same way six other groups of flies, five in each, were tested after having one eye dark adapted, but for various lengths of time. The results obtained in all these tests are presented in Figure 1.

As is shown in this figure, upon exposure to darkness sensitivity to light in the eye of *Eristalis tenax* increases to a maximum in about 60 minutes and remains at about the same level for the following 2 hours, after which it decreases rapidly. It is probable that the ap-

parent variations after adaptation for 90, 120, and 150 minutes are not significant. The drop in the curve at 4 hours is significant, as is shown in the following experiment.

A normal insect with both eyes uncovered was tested in the field of light described in the foregoing after having previously been treated exactly as were the animals whose reactions have been described. In this test it made an average angle of deflection of 3.2 degrees toward the right eye. The left eye was then dark adapted for 60 minutes, and in the test after this period it deflected toward the dark adapted eye at an angle of 26.5 degrees. The left eye was again dark adapted for 60 minutes and in the test following it deflected at an angle of 21.7 degrees in the same direction. Three other tests were given consecutively in which the left eye had been dark adapted for 60 minutes before each test. In the first two the animal deflected toward the side of the dark adapted eye at angles of 13.2 and 10.5 degrees, respectively, while in the third it deflected in the opposite direction at an angle of 30.2 degrees. The results of this experiment support the conclusion drawn in the preceding, that the sensitivity of the eye to light drops rapidly following dark adaptation for 3 hours, and it indicates that the sensitivity of the dark adapted eye becomes even less than that of the light adapted eye.

The results presented do not however indicate the magnitude of the change in sensitivity correlated with dark adaptation. The following tests throw some light on this problem. Nine normal insects with both eyes uncovered were each given tests in the field of light described after having received exactly the same preliminary treatment given those flies used in Exp. I, previously described. The average angle of deflection of all these insects in thirty-six trials was 0.48 degrees toward the side of the left eye (Table II, Exp. III). After both eyes had been thoroughly light adapted for 60 minutes the right eye was dark adapted and the left eye light adapted for 60 minutes in an illumination of 52.9 m.c. The insects were then tested in a field of light in which the luminous intensity in the right beam was 52.9 m.c., while that in the left beam was 21.8 times as intense. Under these conditions the average angle of deflection for all the insects was 5.27 degrees toward the side of the left eye (Table II, Exp. IV). This angle is not greatly different from that made by

the same insects with both eyes equally dark adapted in two beams of light of equal luminous intensity. This shows that it is necessary to increase the intensity of the left beam about twenty-one times in order to overcome the deflection toward the side of the dark adapted eye, indicating that the sensitivity of an eye dark adapted for 60 minutes is about twenty-one times that of the light adapted eye.

TABLE II

RELATIVE SENSITIVITY TO LIGHT OF LIGHT AND DARK ADAPTED EYES OF *Eristalis tenax**

EXPERIMENT	AVERAGE ANGLES OF DEFLECTION IN FOUR TRIALS									TOTAL AVERAGE ANGLES
	Designation of flies									
	f	g	h	i	j	k	l	m	n	
III.....	7.0 *r*	0.2 *r*	5.2 *l*	4.5 *r*	0.5 *r*	15.2 *l*	1.2 *l*	6.5 *l*	11.5 *r*	0.48 *l*
IV.....	8.0 *l*	11.5 *l*	14.0 *l*	2.0 *r*	15.5 *l*	9.5 *r*	10.5 *l*	2.0 *l*	2.5 *r*	5.27 *l*

* *r*, deflection toward right; *l*, deflection toward left: Exp. III, test in two horizontal beams crossing at right angles, illumination in each, 52.9 m.c.; Exp. IV, test in two horizontal beams crossing at right angles, illumination, right beam, 52.9 m.c., left beam, 52.9 by 21.8 m.c. For procedure preceding each experiment see text.

SUMMARY

During exposure to darkness sensitivity to light in the eye of *Eristalis tenax* increases in about 60 minutes to a maximum of about twenty-one times its sensitivity when adapted to a luminous intensity of 52.9 m.c., remains at about this level for 2 hours, then decreases rapidly to a point which is probably lower than that which obtains in light adapted eyes.

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THE EFFECT OF REFRIGERATION UPON THE GROWTH-PROMOTING POWER OF RABBIT TISSUES FOR PLANARIAN WORMS

(One figure)

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A GROWTH-PROMOTING substance present in certain animal tissues, and necessary for the nutrition of planarian worms (Wulzen, 1923) as well as certain other animals (McCay, 1927), has been found to be extremely thermolabile (Wulzen, 1926). Even exposure to a temperature of 37.5°C. for twenty-four hours was found to be sufficient to reduce greatly the growth-promoting power of frog, turtle, and rabbit liver for planarian worms.

Using the method developed by Wulzen (Wulzen, 1926), I have found that refrigeration also has a marked deleterious action upon the growth-promoting power of certain rabbit tissues for planarian worms.

The worms used for all experiments were 6, 7, and 8 mm. in length at the beginning of the experiment, and were from recently regenerated tails. Each experimental group consisted of thirty measured worms, and the total length of an experimental group was used as the unit for the calculation of gains or losses in growth. The worms were fed twice a week, and all experiments were of three weeks' duration. The graphical representation of the experimental results appears in Figure 1. Each column in a series shows the gain in millimeters of a different experimental group of thirty worms.

The tissues used were liver, heart ventricle, lung, and skeletal muscle from the Belgian hare. The fresh tissues were fed immediately upon removal from the body, and samples of the same tissues were placed in small vials, very loosely corked, and deposited in the cold storage plant. Experiments upon liver were conducted at two temperatures. One cold storage plant maintained a temperature from $-1°$ to $0°$ C., while the other held a temperature from $1°$ to $5°$ C.

All of the tissues used were well eaten by the worms. They gathered upon all of the portions of tissue quickly and did not move away until they were distended with the food that they had eaten. Each of the tissues used was also tested in the fresh state by placing a very small portion in a dish with a large number of worms. In each case the tissue disappeared, with the exception of a few strands of white connective tissue, to find which one had to search the dish

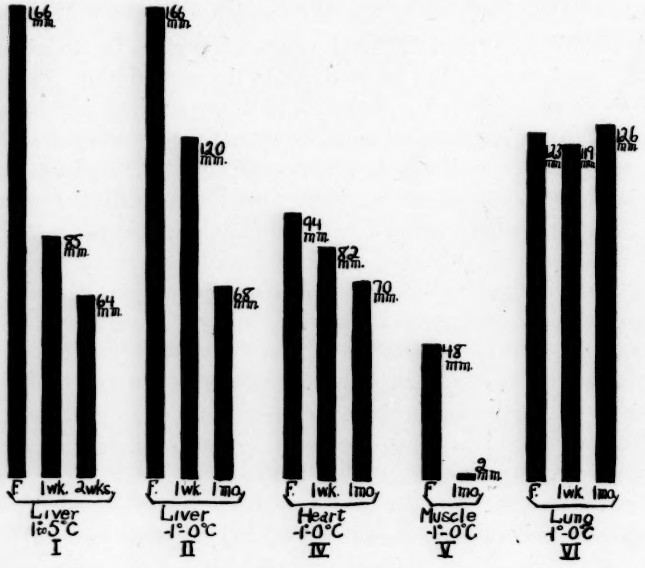

FIG. 1.—Graphic representation of the total gains in millimeters of experimental groups, consisting of thirty planarian worms each, fed upon fresh and refrigerated rabbit tissues. Each column represents a separate experiment, and *F* signifies fresh tissue.

carefully. During the course of refrigeration, the tissues of course become softer, and are therefore even more easily eaten by the worms than when fresh.

Series I (Fig. 1) demonstrates the deleterious action of refrigeration at from 1° to 5° C. upon the growth-promoting power of liver. The total gains of the three experimental groups were as follows:

worms fed fresh liver, 166 mm.; liver refrigerated one week, 85 mm.; liver refrigerated two weeks, 64 mm.; while the percentage gains were respectively 80 per cent, 41 per cent, and 30 per cent.

The loss in the growth-promoting power of liver was not so rapid when the tissue was refrigerated at from −1 to 0° C. as when the temperature was maintained at from 1° to 5° C. (see Fig. 1, Series I and II). The worms fed fresh liver gained 166 mm., or 80 per cent; liver refrigerated 1 week at from —1° to 0° C., 120 mm., or 57 per cent; liver refrigerated 1 month at from −1° to 0° C., 68 mm., or 32 per cent.

Series III, not represented in Figure 1, constitutes an additional experiment on liver maintained at from −1° to 0° C. The loss in growth-promoting power involved here is very similar to that demonstrated by Series II. The total gains in millimeters were: worms fed fresh liver, 227 mm.; liver frozen two weeks, 167 mm.; while the percentage gains were, respectively, 108 and 80 per cent.

The effect of refrigeration upon heart ventricle is shown in Series IV (Fig. 1). Fresh heart tissue did not produce a growth equal to that produced by liver. The fact that different native tissues do contain different amounts of the growth-promoting substance for planarian worms has already been demonstrated by Wulzen (Wulzen, 1923). Worms fed heart ventricle showed the following total gains: fresh ventricle, 94 mm.; ventricle refrigerated one week, 82 mm.; ventricle refrigerated one month, 70 mm.; while the percentage gains were, respectively, 45, 39, and 32 per cent. The refrigeration temperature was from −1° to 0° C.

Although the growth-promoting power of striated muscle is already very low, refrigeration for one month reduced it to a mere maintenance value. When muscle was used in the fresh condition, the experimental group gained 48 mm., or 23 per cent, while the group fed upon muscle refrigerated for 1 month at from −1° to 0° C. gained but 2 mm., or 0.9 per cent (see Fig. I, Series V).

In striking contrast to all these results are the experiments upon lung tissue (Fig. 1, Series VI). Experimental groups fed upon this tissue gained as follows: fresh lung tissue, 123 mm., or 59 per cent; lung tissue refrigerated one week, 119 mm., or 58 per cent; lung tissue refrigerated one month, 126 mm., or 58 per cent. Thus there

has been no material loss in the growth-promoting power of lung tissue after refrigeration at from $-1°$ to $0°$ C. for one month.

SUMMARY

1. Refrigeration at from $-1°$ to $0°$ C. for from one week to one month has been found to reduce the growth-promoting power of rabbit liver, heart, and skeletal muscle for planarian worms.

2. Refrigeration at from $1°$ to $5°$ C. was found to reduce the growth-promoting power of liver much more rapidly than refrigeration at from $-1°$ to $0°$ C.

3. Refrigeration of rabbit lung at from $-1°$ to $0°$ C. for from one week to one month has been found to have practically no deleterious effect upon the growth-promoting power of this tissue.

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THE EFFECTS OF DIFFERENCES IN THE APPARENT SOURCE OF ILLUMINATION UPON THE SHADE ASSUMED BY A FLATFISH ON A GIVEN BACKGROUND

(Two figures)

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I. INTRODUCTION

THE senior author, in 1911, published the results of a rather extended series of observations upon the pigmentary changes which occurred in various species of flounders in response to changes in the optical properties of their background.[1] In the course of these studies various problems of considerable theoretical interest were encountered, one of which was discussed at some length, although the experimental evidence on the subject was not at that time decisive. The nature of this problem may best be indicated by quotations from the earlier article.

In discussing the experiments upon *Rhomboidichthys*, I pointed out that the degree of illumination of the background had little or no effect upon the reaction which the fish underwent. As a special illustration of this principle, it was shown that a fish became paler upon a white bottom, even though this was heavily shaded, than upon a gray bottom exposed to a considerable measure of light. The latter surface, in my experiments with *Rhomboidichthys*, was shown photographically to be very much lighter than the former. Identical results were obtained with *Lophopsetta*. . . . .[2]

It is plain that, in order that the change of shade on the part of the fish should be *adaptive*, the latter would have to behave exactly as described. When, however, we begin to inquire as to the visual stimuli responsible for the changes just recorded, the case becomes decidedly puzzling. For anyone with any knowledge of optics knows that gray—at least a perfectly neutral gray—is not a color. . . . . It differs from white only in this, that it reflects a smaller fraction of the total quantity of light which falls upon its surface. Gray is thus relatively darker than white, but not always absolutely darker. When we ourselves judge

[1] F. B. Sumner, Jour. Exp. Zoöl., X (1911), 409–505, Plate 1–13.

[2] This phenomenon had been recorded both for fishes and crustacea by certain earlier observers.

of an object as being gray or white we make an allowance for the degree of illumination to which it is subjected, and this last is inferred from the totality of the visual field.[1]

But how about the fish? It is not in the position of an outside observer, with abundant standards of comparison at hand. This tank, with its painted surfaces, would seem to constitute for the time being its entire environment. How, then, if the walls of the shaded white box reflect absolutely less light to the animal's eyes than do those of the brightly lighted gray box, does the creature take on a lighter shade in the former than in the latter?

So far as I can see, we are limited to two alternative explanations: either (1) the fish takes into account the degree of illumination, just as we do, and makes due allowance for this in judging of the paleness or darkness of the background; or (2) it makes a direct visual comparison between its own surface and that of the background and endeavors to bring the former into harmony with the latter. [Any possible "anthropomorphic' construction of these words was disclaimed in a footnote]. In this second case, since the body of the fish itself is lighted or shaded to an equal extent with the background, it would have to become fully white in order to conform even to a dimly lighted white background [*op. cit.*, pp. 460–63].

The first of these alternative interpretations of the phenomena in question was later stated as follows:

From the fish's point of view, the only other element of the visual field, besides the bottom and walls of its tank, is the illuminated area overhead, representing the source of light—commonly sunlight reflected from objects outside the tank. May not, then, the ratio between the light reflected from the nearby surfaces within the tank and the light which enters the latter from above be that factor of the total stimulus which renders possible these accurate adjustments of the shade of the fish's body to that of its background?[2] I think that this is the true solution of the problem, and I hope, with apparatus already constructed, to put the question to experimental test, as soon as material is available [p. 477].

The experimental test here referred to was attempted, somewhat later, though unsuccessfully. The chief disturbing element was the presence of vegetable detritus and other opaque substances suspended in the water-supply of the laboratory. These settled upon the clear glass plates upon which the fishes rested, and soon rendered

[1] That even the human observer may be utterly deceived in this matter was well illustrated in the course of the experiments to be described here. Under certain conditions, a gray plate appeared to us and to several other persons as plainly white, and the illusion was corrected only by inserting an actual white plate alongside of it.—S. AND K.

[2] In this idea of reaction to a *ratio*, I was anticipated by Keeble and Gamble (*Phil. Trans. Roy. Soc.*, Series B, Vol. CXCVI [1904]).—*F. B. S.*

them far from transparent. But the plan to put this problem to an experimental test was never entirely abandoned, and it was finally brought to a successful issue during the last year.

The experiments here to be described relate to the first of the alternative hypotheses stated above. Before proceeding to discuss these experiments we must dispose of the other hypothesis mentioned, namely, that the fish makes a direct visual comparison between its own surface and the background.

In the earlier article (Sumner, 1911) certain tests of this visual comparison idea were discussed. These consisted of (1) covering the entire fish, or a part thereof, with black or white cloth, attached to the body by stitches; (2) staining those areas of the skin which fell within the fish's field of vision; (3) incidental observations on fishes which were completely buried in sand, except for the eyes.

The results of the first two sets of tests were not wholly consistent among themselves, and they were partially invalidated by the abnormal conditions which were introduced. On the whole, they did not support the visual comparison hypothesis. But observations upon the behavior of one fish which persistently covered itself with sand furnished strong evidence against this hypothesis. The fish in question was transferred from a white background, upon which it had become extremely pale, to very dark sand. In spite of its burying itself completely, barring the stalked eyes, and of remaining completely buried (except possibly at night) the fish assumed the customary dark shade, probably with normal rapidity. A repetition of the experiment gave identical results.

Mast,[1] who does not regard these last observations as decisive, owing to the possibility that small areas of the fish's head may have remained exposed to view, made similar tests under more rigid conditions. He removed one eye from his specimens and kept the entire head, except the remaining eye, carefully covered with sand. He obtained the same result, however, namely, that the fish underwent the usual adaptive change, regardless of its being unable to observe its own skin surface.

Before discussing our own recent experiments, we shall consider

[1] *Bulletin United States Bureau of Fisheries* (published 1916), XXXIV, 173–238, Plates XIX–XXXVII.

briefly Mast's tests of the alternative hypothesis, that of reaction to a ratio between the source of light and the light reflected from the substratum. Mast employed two types of apparatus, one in which the brightness of the background (substratum) could be increased, while the light from above (apparent source of illumination) could be diminished or cut off altogether; and another, in which the light falling upon the fish's eyes from above could be greatly increased, without the background being affected by this source of illumination.

In experiments with the former apparatus, the fish was placed in a circular glass dish surrounded by a tall cylinder of opaque material, which could be covered above by a dark cloth. Sheets of translucent white or gray paper were inserted beneath the glass dish, and light was reflected from below by a mirror. A number of interesting results were obtained in the course of these experiments, of which the following are of chief interest at present: (1) fishes assumed the maximum degree of pallor under these conditions of illumination, even upon gray paper; (2) when the underlying sheet was spotted heavily with india ink, the spots were "ignored" and the fish became uniformly pale, although this same sheet called forth a spotted pattern upon the fish under normal conditions of illumination.

The former of these results is quite in harmony with those which we have obtained with a somewhat different type of apparatus, and supports directly the "ratio" theory discussed above. The second is likewise of interest in showing the influence of the overhead illumination, even in responses to patterns.

In Mast's second type of apparatus, a similar glass dish and opaque cylinder were employed, but light was not admitted from below. The white background was but feebly illuminated, owing to the opaque cylinder surrounding it, which was covered at the top by cloth. Under these conditions, taken by themselves, the fish would be expected to assume the maximum degree of pallor, as indeed it did. Mast now poised a vertical tube over the fish, in such a way that light, reflected through this from above by a mirror, reached the animal's eyes without appreciably illuminating the white background. One's first belief would naturally be that the "ratio" theory called for a darkening of the fish under these conditions. But such did not occur. The animal, if already of maximum pallor, remained

so. In fact, "in all of these tests the fish retained the shade it had at the beginning of the increase in illumination from above, no matter whether it was dark or light or mottled." This Mast explains on the ground that "the excessive illumination of the eyes from above entirely prevented stimulation by light reflected from the white background" (*op. cit.*, p. 213).

To this defect in technique, fully recognized by Mast, we may add a second one, namely that the overhead light was too narrowly localized. The apparent source of illumination, which constitutes the first term of the ratio *direct: reflected light*,[1] certainly comprises a much larger part of the field of vision than was affected by Mast's 3-centimeter (in one case 3-millimeter) tube. It is likely from other experiments (see following) that the entire visual field of the fish, above a certain rather low level, may figure as the "source" of light, in the sense in which the word is here employed.

Despite the admitted inconclusiveness of his second series of experiments (which were repeated with some modifications), Mast believes that "they do throw considerable doubt on the validity of this [the ratio] hypothesis." While recognizing that "the effect of the light received from the background must be modified, in some way, by light received from some other source," he concludes that "this modification is, however, in all probability, not so simple as is demanded by the hypothesis in question" (p. 214).

II. ACCOUNT OF PRESENT SERIES OF EXPERIMENTS

For these experiments, a small species of flounder, *Hypsopsetta guttulata* (Girard), belonging to the turbot group, was employed. This happens to be the most available local species. The specimens used were seined in False (Mission) Bay, San Diego. These were young individuals, mostly 10 centimeters or less in length. The capacity of this species for chromatic adjustments proved, however, to be rather limited in comparison with the forms previously used either by the senior author or by Mast.

This limitation was found to be especially marked in its reactions to a white background. While such a surface commonly called forth

[1] This is the ratio, in the form given by Keeble and Gamble. In that given by Sumner (1911), the terms are in reverse order (see above).

ultimately a considerable paling of the fish, following transfer from a darker surface, the immediate effect was frequently an actual darkening. Moreover, the shade eventually attained by the animal was invariably far from white, being at best a buff or pale brown. On black or on gray of medium shade, however, the adjustment was frequently rather striking. Upon bottoms of nearly neutral gray, in particular, the animal itself commonly assumed a neutral gray tone, losing almost every trace of brown or yellow.

Since it seemed possible that a pure white surface led to an overstimulation of the animal, inhibiting its normal tendency toward adaptive response, such surfaces were not employed in any except the preliminary tests. Instead of white, a very pale, nearly neutral gray was used, and this was commonly more nearly approximated by the fish.

One fact, which was evident in the earlier studies, must be reckoned with in any experiments upon color changes in flounders. This is the great individual variability in the capacity for adaptive responses to backgrounds. Some individuals, even those which are apparently otherwise normal, are extremely refractory, and may show but slight change after transfer to a strikingly different background. Such fishes obviously must be rejected as material for experiments of the sort here considered. Any results obtained from them would not be contradictory of positive results obtained from other individuals with a proved capacity for adjustment. They would merely be irrelevant.

As in the case of Mast's experiments, cited above, two different types of apparatus were used, designed to produce opposite conditions of stimulation. In the first of these, the substratum was brightly illuminated by light which entered the tank below the level of the fish, while the apparent source of illumination (above the fish) was feeble. In the second case, the light entered the tank above the level of the fish, while the substratum was inclined away from this at such an angle as to be shaded.

Figures 1 and 2 represent in cross-section the apparatus employed in the two cases. The aquarium tanks here used were rectangular, 40 by 25.5 centimeters in horizontal dimensions, and 25.5 centimeters high (interior measurements). The fish, in each case, rested

upon a transparent glass plate, raised about 9 centimeters from the bottom of the tank. To confine it to the central region of this plate

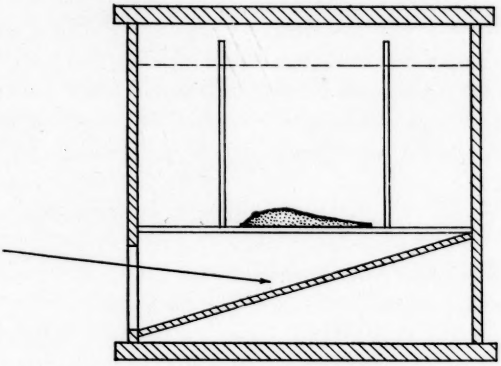

FIG. 1

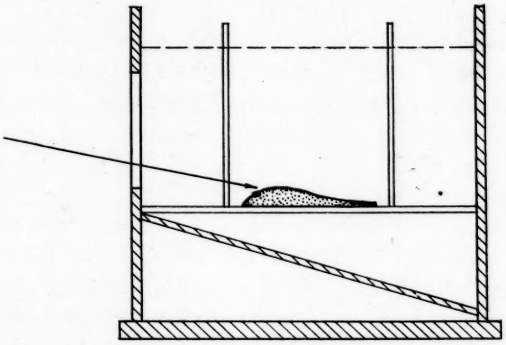

FIG. 2

FIGS. 1 AND 2.—Sectional views of experimental tanks No. 1 and 2, respectively. Shaded areas denote painted glass or other opaque material; unshaded areas denote transparent glass. The inclined plates are the "backgrounds," darker gray in Fig. 1, pale gray in Fig. 2. Arrows denote course of light from window. Fish are on transparent glass plates, surrounded by cylinders of transparent glass. Running salt water, in each case, was supplied fish by a tube entering cylinder from above (not represented).

the fish was surrounded by a cylinder of transparent glass, 13 centimeters in diameter and 15 centimeters high. Sea water was circulated through these cylinders by tubes, not here figured. The glass

walls of the aquarium tanks, with the exception of the clear areas to be mentioned here, were rendered opaque by gray paint, of the darker of the two shades used upon the "background" plates.

The two pieces of apparatus differed from one another in the following respects: in the first case (Fig. 1), the transparent area in the wall of the outer tank was *below* the level of the fish, while the "background" plate, here of the *darker* shade of gray, was tilted *toward* the light. Light was excluded, wholly or partly, from overhead by a movable wooden cover.

In the second case (Fig. 2), the transparent area was *above* the level of the fish, the "background" plate was coated with the *pale* gray paint, and was tilted *away from* the light. Likewise the diffused light of the room was allowed to enter freely from above.

The chief source of illumination, in each case, was light from a nearby window, partly covered by a roller shade, so that the rays entered at a plane approximately horizontal.

The lighter and darker gray paints employed for the backgrounds, were very close to the "dawn gray" and "slate gray," respectively, of Ridgway's *Color Standards and Nomenclature*.[1]

Control aquariums, of the same size as the foregoing, were provided with painted plates of these two shades of gray. In the controls, however, the plates were horizontally placed, and rested directly upon the bottoms of the tanks. Likewise the fish were commonly in immediate contact with these painted backgrounds, and both animal and background received light from the same sources, i.e., from above and also (usually) through the side walls of the tank.

In our experiments a fish was transferred from one or another of these "control" tanks, with the bottom of which its color had come into harmony, to one or another of the "experimental" tanks. The occurrence and the extent of any resulting change of shade were determined in one of two ways. (1) The fish, after a sufficiently long sojourn in the "experimental" tank (from one to several days), was quickly removed to the "control" background, from which it had

[1] These comparisons have been made with the paints when seen through the glass plates (as was done by the fish) and when dry. After soaking in sea water they became somewhat paler in appearance, and the rust in the water-supply of the station gave rise to a slight brownish deposit which had to be removed from time to time.

been taken, and compared with the latter and with "control" fishes which had become well adjusted to this background. (2) A fish from the proper "control" tank was transferred to the "experimental" one, and compared with the fish in question, before the "control" fish had time to change.

According to the hypothesis which we are testing, the following results would be expected from the four possible types of experiment:

1. From darker gray control background to experimental tank No. 1: *fish should become paler.*

2. From pale gray control background to experimental tank No. 1: *fish should remain pale.*

3. From darker gray control background to experimental tank No. 2: *fish should remain (relatively) dark.*

4. From pale gray control background to experimental tank No. 2: *fish should become (relatively) dark.*

All four of these tests were carried out, as follows: (1) fourteen trials, (2) two trials, (3) one trial, (4) four trials. Only four of the trials gave negative results, all of these being in Test 1.

Ten fishes were actually employed in these experiments, a number of others being rejected, owing to their limited capacity for color change. Of the ten experimental fishes, eight gave only results of the "expected" type, while two gave only negative results. The latter were both poor "performers," which remained distinctly darker than their associates upon the control backgrounds. Their behavior cannot be regarded as seriously weakening the positive evidence.

It was necessary, of course, to eliminate the possibility that the results obtained in the experimental tanks were affected in any important way by the elevation of fishes above these backgrounds. To test this matter a plate of clear glass was raised above the gray bottom in one of the control tanks. The distance (about 3.5 centimeters) was approximately the mean distance between the transparent glass plate and the background in the experimental tanks. Two of the fishes used in the main series of experiments were tested for this purpose. The results gave satisfactory evidence that elevating above the background did not affect the shade of gray assumed.

Likewise, another fish was placed in experimental tank No. 1, with the lower window covered, so that illumination was entirely

from above. The reaction was that ordinarily called forth by the darker gray, until light was admitted from below, whereupon the fish rapidly paled.

Attention should be called to the relative position of the fish and of the source of illumination in Figure 2, which has been drawn approximately to scale. It is evident that most of the light rays reaching the flounder fell within an angle of less than 45 degrees with the glass plate on which the latter rested. Thus the "source" of illumination (the "direct" light of the ratio) may here have comprised all the light that reached the fish outside of a rather small angle with the horizontal.[1]

To sum up the results of these experiments, it may be stated that, barring the behavior of a few refractory individuals, whose adjustments were poor under any circumstances, the responses were such as would be called for by the "ratio" hypothesis discussed in the foregoing. A background of moderately dark gray, brightly lighted from below, in conjunction with a feeble apparent source of illumination, evoked the maximum degree of pallor; while a much paler background, inclined so as to receive but little of the light which fell upon the fish from above the level of its eyes, called forth a distinctly darker condition.

It should be explained, however, that this "ratio" principle is not here construed in any strictly quantitative sense. It is merely maintained that, speaking generally, the shade assumed by the fish depends directly upon the intensity of the light reflected from the substratum ("background") and inversely upon the intensity of the light received from above (outside of a certain angle with the horizontal).

[1] That portions of the fish's field of vision above the level of its body may be comprised in the effective "background," and influence the animal's reaction in a positive sense, has been shown by earlier experiments (Sumner, 1911, pp. 451–55).

THE EFFECT OF OXYGEN TENSION ON OXYGEN CONSUMPTION IN PLANARIA AND SOME ECHINODERMS

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THE relation of oxygen consumption to oxygen tension has been examined in a considerable range of organisms, from bacteria to man, with varying results. A brief survey of the literature will serve to indicate the present status of the problem.

In *Bacillus coli communis* (Stephenson and Whetham, 1924), oxygen consumption and carbon dioxide production are much greater in an oxygen atmosphere than in air. The tubercle bacillus responds similarly (Novy and Soule, 1925) but presents the maximum increase at 40 per cent oxygen, with little augmentation at 60 and 80 per cent; decreased oxygen tension (from 6 to 0.5 per cent) is accompanied by a directly proportional decline in oxygen consumed.

Among algae, Wurmser and Jacquot (1923) reported a direct dependence of oxygen consumed on dissolved oxygen in *Laminaria* at concentrations ranging from 5 to 26 cc. per liter. Contrariwise Hée and Bonnet (1925) observed no change in the carbon dioxide production of the fresh water algae *Nitella* and *Cladophora* between 4 and 15 cc. of oxygen per liter, with in some cases a slight increase or decrease above and below these values, respectively. According to the same authors the carbon dioxide production of the mushroom *Sterigmatocystis nigra* is identical in air, 50 per cent oxygen, and pure oxygen; and in a number of aquatic angiosperms (*Elodea*, *Myriophyllum*, *Potamogeton*, *Cabomba*) is constant between 3 and 24 cc. per liter of dissolved oxygen, except for some slight increase at the highest tension. Godlewski (1882) found some dependence of oxygen consumption on oxygen tension at low tensions in germinating seedlings and other plant materials; but both Johannsen (1885) and Stick (1891) failed to observe an alteration in carbon-dioxide production at different oxygen tensions in various seedlings, fruits, flowers, buds, etc.,

except at very low concentrations. In general the oxygen consumption and carbon-dioxide production of plant materials appears to be independent of oxygen concentration.

The data on animals are so numerous and varied that it seems best to present them under the various phyla.

Protozoa.—The oxygen consumption of *Paramecium* is constant over a wide range of oxygen concentration (Lund, 1918; Amberson, 1928).

Porifera.—In a sponge, unspecified, a great decline in oxygen consumption was noted in a long as compared with a shorter respiratory period, indicating decreased rate at low tensions (B. Moore *et al.*, 1912).

Coelenterata.—The jellyfish *Pelagia noctiluca* and *Carmarina hastata* are independent of oxygen concentration (Henze, 1910) but *Cassiopaea* exhibits considerable dependence at low tensions (McClendon, 1917). Likewise in the anemones *Actinia equina* and *Anemonia sulcata*, the oxygen consumed depends upon the oxygen tension (Henze, 1910).

Platyhelminthes.—The oxygen consumption of *Planaria agilis* is constant in water whose oxygen content varies from air-saturation to one-third or one-fourth of this value, declines at lower concentrations, and is slightly accelerated in supersaturated water (Lund, 1921).

Echinodermata.—The experiments of B. Moore *et al.* (1912) indicate a lack of relation between oxygen consumed and oxygen content of water in *Asterias* and *Echinus*. The respiration of sea-urchin eggs is likewise independent of oxygen concentration (Henze, 1910; Amberson, 1928). But in the holothurian *Caudina chilensis* (Nomura, 1926), the rate of oxygen consumption is directly proportional to the oxygen tension when the latter declines through the respiration of the animal.

Gephyrea.—Henze (1910) states that in *Sipunculus nudus*, the oxygen consumed depends upon the oxygen concentration.

Annelida.—The oxygen consumption of *Nereis virens* is directly proportional to oxygen tension (Amberson *et al.*, 1924) when the latter diminishes through the respiration of the worms. In earthworms, Thunberg (1905) found that any increase in atmospheric

oxygen from the normal 20 to 97 per cent augments the oxygen intake, but Konopacki (1907) observed such an increase only up to 50 per cent oxygen, with a constant rate above that value. Different species may, however, have been employed. The effect of lower tensions does not seem to have been studied in earthworms.

Mollusks.—All aquatic mollusks tested maintain their normal oxygen intake at reduced tensions, at least down to a certain minimum: *Anodonta* (Dakin and Catherine, 1925), *Buccinium*, *Aplysia*, and *Fusus* (B. Moore *et al.*, 1912), *Loligo pealii*, constant to 30 per cent saturation (Amberson *et al.*, 1924). Henze (1910) reported a constant rate at increased tensions also in *Aplysia* and the cephalopod *Eledone*. In *Limax agrestris*, a terrestrial slug, the oxygen consumption is lowered at 5 to 15 per cent oxygen and increased at 50 and 97 per cent (Thunberg, 1905).

Arthropoda.—The oxygen consumption of *Carcinus moenas* and *Scyllarus latus* is independent of oxygen content over a wide range (Henze, 1910); as is also that of *Palaemonetes* between air-saturation and 50 per cent saturation (Amberson *et al.*, 1924). The latter authors, however, find direct dependence of oxygen consumed on oxygen content in *Homarus americanus*, *Limulus polyphemus*, and *Callinectes sapidus*, when the oxygen supply decreases through the respiration of the animals. The data of B. Moore *et al.* (1912), indicating dependence in *Cancer* and *Eupagurus*, are of doubtful value as the oxygen supply was probably exhausted long before the termination of the experiment. Crayfishes have been tested by several workers, with rather variable results, perhaps partly attributable to the employment of different species. Brunow (1911) reported a continuous decline in oxygen intake in *Astacus fluviatilis* as the oxygen was exhausted but Helff (1928) has found the oxygen consumption of *Cambarus immunis* constant down to 20 to 30 per cent of saturation. Hée and Bonnet studied the oxygen consumption of *Astacus fluviatilis* at concentrations varying from 5 to 22 cc. of oxygen per liter; the rate was usually constant between 5 and 8 cc. but increased with increasing oxygen content above the latter value. The pupae of *Tenebrio* respire at a constant rate in atmospheres containing a percentage of oxygen varying from 5 to 97 (Gaarder, 1918), but at 20° C. show some decline at percentages below 5. *Tenebrio* larvae (Thun-

berg, 1905) also exhibit decreased oxygen intake in atmospheres containing 10 per cent or less of oxygen.

Chordata.—The respiration of vertebrates in general is highly independent of oxygen tension. Fish allowed to exhaust the oxygen content in closed vessels maintain a constant rate down to very low tensions: goldfish (Dakin and Catherine, 1925), *Blennius* (Moore *et al.*, 1912), *Fundulus* (Amberson *et al.*, 1924). Similar results obtain over wider ranges of oxygen content, as shown by Henze (1910) for *Coris* and *Sargus*, Winterstein (1908) for *Leuciscus*, whose rate of oxygen intake is the same at 2.3, 5.9, and 28.7 cc. of oxygen per liter, and Hée and Bonnet for the tench, employing variations in oxygen content from 3.79 to 26.07 cc. per liter. In the carp, however, the oxygen consumption increases slowly with rising oxygen content (Gaarder, 1918), but the change is so slight as to be negligible over small ranges. The oxygen intake of plaice eggs in closed vessels was stated by Dakin and Catherine (1925) to diminish with time; but Burfield (1928) subsequently demonstrated that this diminution resulted from the accumulation of carbon dioxide around the eggs and could be eliminated by agitating the eggs frequently. The oxygen consumption of axolotls remains constant at decreased oxygen content down to a certain minimum (Dakin and Catherine, 1925). Pott (1883) noted an increased carbon-dioxide output beginning with the second week of incubation in developing hen's eggs kept in an atmosphere of oxygen as compared with air. In man, low barometric pressures (410 to 310 mm. of mercury) have little effect on oxygen intake at least during short exposures (Schneider *et al.*, 1924).

The foregoing survey reveals a lack of uniformity, except as regards vertebrates. Vertebrates in general consume oxygen at a constant rate regardless of the oxygen content of the medium, perhaps because of their possession of efficient oxygen-carrying mechanisms. But in invertebrates the relation of oxygen intake to oxygen concentration in the medium is highly variable in different forms and exhibits no correlation with taxonomic position, complexity or simplicity of organization, or presence or absence of oxygen-carrying compounds. Closely related forms may respond quite differently to changes of oxygen tension as, for instance, in the decapod Crustacea, where some crabs are found to consume oxygen without regard to

oxygen tensions while others display a marked dependence on this factor. Whether such dependence or independence is related to the mode of life of the animal cannot be determined from the available data, as most investigators have failed to consider this matter. One might suppose, for instance, that animals, such as pelagic forms, which are always exposed to an abundance of oxygen, would be incapable of maintaining their normal oxygen intake at low oxygen tensions. But reference to the literature fails to support this supposition since some jellyfish, for instance, are found to be quite independent in their respiration of oxygen tension while others reveal a dependence. Similarly, one might assume that bottom-dwelling forms, living in an environment tending to be low in oxygen content, would be so adapted as to be able to respire at a constant rate regardless of tension; but while the data on one bottom-living form, such as *Anodonta*, support this surmise, those on others, such as *Nereis*, fail to conform. Up to the present no adequate explanation of the situation has been forthcoming. No general law can be stated and it becomes necessary in the case of each species used for respiratory studies to determine its response to changes in oxygen tension, unless the range of tension involved is small.

From the literature it appears that in animals the relation of oxygen consumption to oxygen concentration is of three different types. Some animals are quite or nearly independent of oxygen tension, e.g., *Paramecium*, fish. Others are able to respire at a constant rate over a certain range of oxygen concentration but are affected at tensions greater or less than this, as for instance, the crayfish and *Planaria*. In a third category fall those forms whose oxygen intake is at each and every oxygen concentration dependent upon that concentration, as the lobster and *Nereis*.

The possibility remains that the method of experimentation may affect the result in some instances. Many workers have studied the effect of low tensions by allowing the animal to exhaust the oxygen in closed containers through its own respiratory activity. Under such conditions, there is an accumulation of carbon dioxide and other waste substances, whose presence may modify the result as demonstrated in the case of plaice eggs. Amberson and his co-workers (1924) have, however, considered the question of the accumulation

of carbon dioxide (with every animal?) and state that this factor was not concerned in their results. I have, however, shown in *Planaria* that the presence of carbon dioxide in sufficient amount does lower the rate of oxygen consumption regardless of the oxygen content and further that a combination of high carbon dioxide and low oxygen content is more effective in inhibiting oxygen intake than either factor alone (Hyman, 1925). Whether animals respiring in a closed vessel would produce enough carbon dioxide to affect their oxygen intake would have to be determined for each species and would also depend upon the mass of the animal relative to the quantity of water. I shall show here that in *Planaria* different modes of procedure yield diverse results, but the difference is not in the direction which would be anticipated on a priori reasoning.

MATERIAL AND METHOD

The starfish *Patiria miniata*, the sea-urchin *Strongylocentrotus purpuratus*, and the fresh-water triclad *Planaria dorotocephala* were used as materials. The two marine forms were experimented on at the Hopkins Marine Station, Pacific Grove, California. I desire to express my thanks to the director, Professor W. K. Fisher, for placing the resources of the laboratory at my disposal. The work on the planarian was performed at Chicago.

The method was the same as previously employed in oxygen-consumption work. The animals were placed in 500 cc. Erlenmeyer flasks or in the case of the echinoderms in wide-mouthed salt bottles of from 500 to 1,000 cc. capacity. The water to be used was placed in a large elevated receptacle from which it was siphoned into the flasks or bottles containing the animals. The bottles were closed air-tight and the animals allowed to respire in them for an arbitrary time interval, generally one hour. A sample was then drawn by siphon into an approximately 125-cc. bottle, which was analyzed for oxygen content by Winkler's method. At Pacific Grove, the sea water used was carried directly from the bay. At Chicago, the well water in the Hull Zoölogical Laboratory was employed. The oxygen content was increased when desired above air-saturation by bubbling oxygen from a tank through some or all of the water used. Decreased oxygen tension was secured by boiling for a few minutes all or part of the

water. By proper mixing, various tensions can be secured, although it is not easy to obtain any specific tension. This, however, was not necessary.

The procedure in any one experiment was as follows: The animals were placed in the containers some time before the experiment was begun in order to reduce their activity. Two successive determinations in air-saturated water were then made, the water being changed for each determination. Water of the desired oxygen tension was then prepared and another determination made of the oxygen consumption in such altered water. In some experiments, the oxygen tension was raised or lowered gradually by successive steps over a period of several determinations. In others, the oxygen content was kept at very high or very low values for several successive determinations. It is to be understood that fresh water is prepared for each hourly determination, except in one series with *Planaria*, in which the animals were allowed to decrease the oxygen content through their own respiration.

EXPERIMENTS WITH PLANARIA

1. *General procedure.*—Animals, 20–25 mm. long, starved about four days, were selected from the laboratory stocks and placed in 500 cc. Erlenmeyer flasks the day before the experiment was begun. This procedure obviates the restlessness induced by placing worms in unfamiliar clean containers. Three lots, each consisting of from 100 to 150 worms, were generally run simultaneously. Such lots were used for about a week without feeding, whereupon they were returned to the general stocks, and new lots, starved four days, selected. Any effects of either feeding or starvation on oxygen consumption were thus avoided. The well water was heated but not boiled on the day before utilization to rid it of excess carbon dioxide. After standing over night it was brought to air-saturation with oxygen by shaking it violently in a bottle. The pH of such water was from 8.0 to 8.2, a value which yields a "normal" rate of oxygen consumption (cf. Hyman, 1925, Anderson, 1927). Addition of oxygen gas to such water or boiling did not alter the pH. The carbon dioxide given off by the worms in one hour produces only a slight alteration of the hydrogen-ion concentration. The greatest acidity noted after inclo-

sure of worms in such flasks for five hours was pH 7.7. Such a slight increase in hydrogen-ion concentration when due to carbon dioxide does not affect the oxygen consumption of *Planaria*, as previously shown (Hyman, 1925). The temperature of all experiments was 20° C.±0.5 and the contents of the flasks and sample bottles were determined at, and the volume of oxygen consumed calculated for, this temperature. The thiosulphate was standardized at frequent intervals with freshly resublimed iodine.

2. *Respiration for one hour at increased oxygen tensions.*—In this series the oxygen consumption of given lots of worms was determined for two successive hour periods in air-saturated water (oxygen content, from 6 to 7 cc. per liter at 20° C.). The oxygen intake of the same lots was then measured for one hour in water whose oxygen content had been raised to varying amounts by the addition of oxygen gas. The oxygen content was increased in different experiments over a range varying from about 8 to 21 cc. per liter. A number of typical experiments are presented in Table I and all of the experiments are summarized in Table II. The data are arranged according to increasing oxygen content.

Inspection of both tables shows that the oxygen consumption of *Planaria* is in the majority of instances not affected by raising the oxygen content of water, until the oxygen content reaches a concentration of something like 17 cc. per liter. The oxygen consumption is constant up to 9 cc. per liter. Between 9 and 17 cc. per liter some lots (15 per cent) may show an increase in oxygen consumption as in experiments 40*b* at 10.34 cc. per liter, 23*c* at 12.34 cc. per liter, 43*a* at 13.71 cc., and 43*b* at 16.14 cc., in Table I. Most lots at these same concentrations do not exhibit any change in oxygen consumption. Between 17 and 21 cc. of oxygen per liter, the majority of worms respond by increased oxygen intake, but even at these high concentrations, some lots retain the same oxygen consumption as at 7 cc., as, for instance, Nos. 9*c*, 19*a*, and 28*a* in Table I.

The oxygen consumption of *Planaria* is thus relatively independent of oxygen tension in supersaturated water. Even where an increased intake is observed, the amount of increase is small and is not proportional to the rise in oxygen tension. Out of a total of 137 experiments at increased tensions, 27 showed augmented oxygen in-

take. Of these, the augmentation lay between 15 and 30 per cent in 22 cases, in only five did it attain 50 per cent, while in one case at

TABLE I

EFFECT OF RAISING THE OXYGEN CONTENT OF WATER ON THE AMOUNT OF OXYGEN CONSUMED BY *Planaria dorotocephala:* HOUR DETERMINATIONS*

No. of Exp.	O_2 Content at Start, Cc. per Liter	O_2 Consumed by Worms, Cc. per Hour	No. of Exp.	O_2 Content at Start, Cc. per Liter	O_2 Consumed by Worms, Cc. per Hour
17*a*	6.60	0.57	27*a*	6.76	0.78
	6.77	0.51		6.82	0.69
	8.03	0.57		13.93	0.73
23*a*	6.67	0.69	7*a*	6.52	0.49
	6.72	0.67		6.48	0.46
	8.43	0.68		15.48	0.47
24*a*	6.69	0.61	43*b*	6.48	0.57
	6.50	0.62		6.49	0.52
	8.80	0.57		16.14	0.73
17*b*	6.60	0.57	18*b*	7.05	0.41
	6.77	0.51		6.92	0.48
	9.06	0.53		17.73	0.46
40*b*	6.61	0.53	23*a*	6.67	0.69
	6.52	0.55		6.72	0.67
	10.34	0.64		18.16	0.82
18*a*	7.05	0.46	9*c*	6.35	0.45
	6.92	0.40		6.42	0.42
	10.67	0.44		19.47	0.47
29*a*	6.66	0.65	19*a*	6.66	0.48
	6.70	0.62		6.61	0.45
	11.41	0.64		20.00	0.50
23*c*	6.67	0.56	19*c*	6.66	0.49
	6.72	0.59		6.61	0.47
	12.34	0.67		20.00	0.61
18*b*	7.05	0.41	28*a*	6.65	0.67
	6.92	0.48		6.70	0.64
	12.97	0.45		20.77	0.67
43*a*	6.48	0.49			
	6.49	0.55			
	13.71	0.65			

* Each experiment gives two successive hour determinations of oxygen consumed at ordinary oxygen tension (from 6 to 7 cc. per liter at 20° C.) followed by an hour determination at raised oxygen content. Temperature of all experiments 20° C.

the highest tension used, an increase of 77 per cent appeared. The average increase as shown in Table II was not more than 35 per cent.

The amount of increase bore no relation to the increased oxygen tension but might be as great at 10 as at 20 cc. per liter concentration.

In considering the tables the amount of variation between two successive determinations at air-saturation, as shown in Table I, should be borne in mind. There is some tendency for the first determination to be higher than others because the worms are more disturbed by the setting up of the experiment at the start than at later changes of water. In general, the animals were very quiet throughout the tests, generally settling down into immobility a few minutes after the flasks were closed.

TABLE II

SUMMARY OF ALL EXPERIMENTS ON THE EFFECT ON OXYGEN CONSUMPTION OF RAISING THE OXYGEN CONTENT OF WATER FOR ONE HOUR

Oxygen Content, Cc. per Liter	7–9	9–11	11–13	13–15	15–17	17–19	19–21
Total no. of experiments	21	25	17	17	9	8	16
Oxygen intake unchanged	20	20	14	14	8	4	6
Oxygen intake raised	1	5	3	3	1	4	10
Min. and max. percentage of rise	17	18–53	16–25	17–52	23	20–32	14–77
Average percentage of rise	17	35	21	31	23	25	33

3. *Respiration in gradually increasing oxygen tensions over three to five hours.*—In this series after the usual two control determinations at air-saturation, the oxygen content was raised by gradually increasing amounts over three to five successive hours. Some typical experiments are shown in Table III and at the bottom of this table is given a general summary of the results. The latter are in general accord with the preceding findings. In some lots of worms, as Nos. 18*a* and 18*b*, the oxygen intake remained constant throughout the experiment. Of a total of 24 experiments, 11, or nearly half, exhibited this behavior. In four other cases, there was no rise in the oxygen consumption but some tendency to fall during the first hours at increased tension with a return to normal later, as in 24*c*, Table III. The other nine experiments exhibited some rise either toward the end in the higher tensions, as in Nos. 24*a* and 43*c*, or a rise during the first hours in raised tensions, followed by a fall, followed by a rise again, as in Nos. 23*a* and 23*b*. In general there seemed to be a considerable tendency to drop back to or below nor-

mal during some of the period at raised oxygen tensions. This is further indicated in the next series.

TABLE III

EFFECT ON OXYGEN CONSUMPTION OF *Planaria dorotocephala* OF GRADUALLY RAISING THE OXYGEN CONTENT OF WATER OVER A PERIOD OF SEVERAL HOURS, WITH HOURLY CHANGES AND DETERMINATIONS*

No. of Exp.	O_2 Content at Start, Cc. per Liter	O_2 Consumed by Worms, Cc. per Hour	No. of Exp.	O_2 Content at Start, Cc. per Liter	O_2 Consumed by Worms, Cc. per Hour
	6.69	0.61		6.67	0.69
	6.50	0.62		6.72	0.67
	7.49	0.64		8.43	0.68
24*a*	8.80	0.57	23*a*	12.34	0.75
	10.47	0.62		13.51	0.61
	11.02	0.67		18.61	0.82
	13.74	0.75			
	7.05	0.46		6.67	0.73
	6.92	0.40		6.72	0.74
	9.26	0.40		8.43	0.80
18*a*	10.67	0.44	23*b*	12.34	0.92
	12.97	0.48		13.51	0.77
	13.91	0.46		18.16	0.97
	17.73	0.47			
	7.05	0.41		6.48	0.57
	6.92	0.48		6.49	0.52
	9.26	0.40	43*b*	8.76	0.61
18*b*	10.67	0.43		13.71	0.63
	12.97	0.45		16.14	0.73
	13.91	0.45			
	17.73	0.46		6.48	0.53
				6.49	0.48
	6.69	0.60	43*c*	8.76	0.59
	6.50	0.55		13.71	0.66
	7.49	0.51		16.14	0.74
24*c*	8.80	0.48			
	10.47	0.61			
	11.02	0.64			
	13.74	0.68			

* In each experiment two successive hour determinations of oxygen consumption at ordinary oxygen tensions (6–7 cc. per liter at 20° C.) are given followed by several hourly determinations at gradually increasing oxygen tensions. Temperature, 20° C.

Total number of experiments, 24; number showing no change in oxygen consumption, 11; number showing no rise, but temporary fall, 4; number showing more or less rise, 7; number showing rise, followed by fall, 2.

4. *Respiration at high oxygen tension for several hours, followed by return to normal tension.*—In this series, presented in Table IV, after the usual two control determinations, the oxygen tension was raised to from 15 to 20 cc. per liter and kept at similar tensions over three or

four successive hours. Usually the tension was then returned to normal and one further test made. Typical cases and a summary of the twenty-one experiments performed are presented in Table IV. The results showed similar variability to the preceding cases. In five of

TABLE IV

EFFECT ON THE OXYGEN CONSUMPTION OF *Planaria dorotocephala* OF MAINTAINING A VERY HIGH OXYGEN CONTENT FOR THREE OR FOUR HOURS FOLLOWED BY RETURN TO NORMAL OXYGEN CONTENT*

No. of Exp.	O_2 Content at Start, Cc. per Liter	O_2 Consumed by Worms, Cc. per Hour	No. of Exp.	O_2 Content at Start, Cc. per Liter	O_2 Consumed by Worms, Cc. per Hour
	6.35	0.43		6.65	0.69
	6.42	0.47		6.70	0.66
	19.47	0.53		20.77	0.80
9*a*	19.12	0.50	28*b*	19.34	0.70
	18.68	0.44		19.54	0.85
	19.67	0.46		6.66	0.62
	6.39	0.43			
				6.65	0.63
	6.35	0.46		6.70	0.63
	6.42	0.43		20.77	0.72
	19.47	0.50	28*c*	19.34	0.65
9*c*	19.12	0.62		19.54	0.87
	18.68	0.48		6.66	0.65
	19.67	0.58			
	6.39	0.41		7.10	0.57
				6.77	0.50
	6.66	0.48	8*a*	15.31	0.56
	6.61	0.45		18.09	0.50
	20.00	0.50		19.01	0.53
19*a*	17.52	0.43		6.36	0.48
	19.30	0.51			
	6.36	0.38		6.27	0.68
				6.26	0.63
	6.66	0.46	4*a*	18.99	0.87
	6.61	0.43		17.70	0.57
	20.00	0.58		17.46	0.62
19*b*	17.52	0.40		10.20	0.67
	19.30	0.53			
	6.36	0.38			

* Total number of experiments, 21; number showing no change, 5; number showing rise, then fall, then rise, 9; number showing rise, then fall, then normal, 3; number showing fall without rise, 2; number showing no change, then rise, then normal, 1; number showing general rise, 1.

the twenty-one experiments the oxygen consumption remained practically constant through the period in high oxygen tensions and on return to normal tension as in Nos. 9*a* and 8*a* in Table IV. In more cases, a total of 9, the oxygen tension showed some rise during the first hour or two at high tension, then declined during the next hour,

and then rose again, as in Nos. 9*c*, 19*b*, 28*c*, and 28*b* in Table IV. In three other cases, the result was similar, except that after the fall the oxygen consumption returned to normal instead of exhibiting a second rise, as in 4*a*. In two cases there was a fall during the high tensions, but no rise, while in one case there was a rise with return to normal and without any fall. In only one experiment was a general rise during the exposure to high tensions observed. The amount of rise was similar to that obtained in the previous cases, being generally moderate.

The tendency already noted for the oxygen consumption to decline during part of the period in high tensions was very marked in this series of experiments. Of the total of twenty-one experiments, sixteen showed an effect of the high tensions and of these all but one exhibited a decline below normal or a return to normal during some part of the period passed at high tensions. Inspection of the table indicates that such fall was usually associated with a slight decline in the oxygen tension. It was found difficult to keep the water at a given tension and there was usually some variability in the tension during the experiment. It thus appears that slight diminutions in tension when working at high tensions have an altogether disproportional effect in lowering the oxygen consumption.

In 18 of the 21 experiments, the worms were returned to water of normal oxygen concentration after three or four hours' sojourn at high tensions. In but 3 of the 18 cases did the oxygen consumption fall decidedly below the normal on return to the normal tension, as in Nos. 19*a* and 19*b* in Table IV. A few others showed some slight effect as perhaps Nos. 9*c*, 28*b*, and 8*a* in Table IV. Most of the experiments did not give any such effect. In general it appeared that in those cases in which there was little or no rise during the period at high tensions a tendency to decline below normal on return to normal water was manifest. This again suggests that the oxygen consumption of *Planaria* is more affected by relative changes in tension than by absolute tension.

5. *Oxygen consumption during one hour at lowered tension.*—After two control determinations at air-saturation, the oxygen content was lowered to various degrees by boiling part of the water used, and the respiration at lowered tension taken for one hour. Typical results

are presented in Table V and all of the experiments are summarized in Table VI. It will be seen from the data in both tables that the oxygen consumption remains constant down to a concentration of about 3 cc. per liter. Below this value the majority of experiments show a diminished oxygen intake although the oxygen supply is still

TABLE V

EFFECT OF LOWERING THE OXYGEN CONTENT OF WATER ON THE RATE OF OXYGEN CONSUMPTION OF *Planaria dorotocephala**

No. of Exp.	O_2 Content at Start, Cc. per Liter	O_2 Consumed by Worms, Cc. per Hour	No. of Exp.	O_2 Content at Start, Cc. per Liter	O_2 Consumed by Worms, Cc per Hour
10*a*	6.29	0.55	42*a*	6.29	0.34
	6.11	0.54		6.31	0.32
	5.72	0.51		3.80	0.35
15*b*	6.44	0.46	36*b*	6.70	0.47
	6.43	0.51		6.71	0.48
	5.39	0.49		2.82	0.44
36*b*	6.70	0.47	12*c*	6.48	0.53
	6.71	0.48		6.48	0.57
	5.10	0.52		2.52	0.45
15*a*	6.44	0.49	42*a*	6.29	0.34
	6.43	0.52		6.31	0.32
	4.67	0.49		2.22	0.26
37*a*	6.50	0.53	39*b*	6.38	0.85
	6.49	0.50		6.45	0.80
	4.41	0.50		1.88	0.56
12*b*	6.48	0.46	39*c*	6.38	0.68
	6.48	0.52		6.45	0.68
	4.24	0.46		1.88	0.66
15*a*	6.44	0.49	13*b*	6.37	0.43
	6.53	0.52		6.51	0.44
	4.13	0.50		1.74	0.31

* Two successive determinations at normal oxygen concentration (6–7 cc. per liter) are given followed by one hour at lowered oxygen concentration. Temperature, 20° C.

ample for normal intake. Practically all experiments at concentrations below 2 cc. per liter yield a decided decrease in oxygen consumption. It should be borne in mind that in Table V the oxygen content in cubic centimeters per liter is given only for the beginning of the test and is reduced about 1 cc. per liter during the test by the respiration of the worms.

The percentage decrease is not very large even at the lowest con-

centrations used, which reached about 1 cc. per liter after an hour's respiration. The decrease in oxygen intake is not proportional to the decline in oxygen concentration but does bear some relation to the latter, as shown in Table VI.

It is concluded then, that by the method employed the oxygen intake of *Planaria dorotocephala* becomes limited by oxygen concentration when the latter declines to a value of about 3 cc. per liter. A similar result was reached for *Planaria agilis* by Lund (1921). It will be shown shortly that when the method is altered a different result is obtained.

TABLE VI

SUMMARY OF ALL EXPERIMENTS ON THE EFFECT ON OXYGEN CONSUMPTION OF LOWERING THE OXYGEN CONTENT FOR ONE HOUR

Oxygen Content, Cc. per Liter at Start	5.7–4.6	4.4–3.8	2.8–2.5	2.2–2.0	1.9–1.7
Total number of experiments	18	18	9	7	12
Oxygen intake unchanged	17	17	5	0	1
Oxygen intake lowered	1	1	4	7	11
Minimum and maximum decrease, per cent	20	14	15–22	15–35	15–44
Average decrease, per cent	20	14	19	21	31

6. *Oxygen consumption during several successive hours in which the oxygen content was gradually lowered.*—In this series, presented in Table VII, after two determinations in normal air-saturated water, the oxygen intake was measured during two or three successive hours in which the oxygen content was gradually lowered. The result is the same as in the preceding series. The oxygen consumption remains constant until the oxygen content falls to a point between 2 and 3 cc. per liter and then declines. It should be mentioned once more that the oxygen content per liter at the end of an hour's respiration will be about 1 cc. lower than the content at the start. Only the latter value is presented in the table. Return to normal water after respiration in low oxygen water is generally accompanied by an increase in oxygen content above the normal, as in Nos. 12*a* and 12*c*. There was a total of 21 experiments in this series, all of which were in accord with the foregoing statements.

7. *Respiration at low oxygen tension for two or three hours, followed by return to normal oxygen content.*—These data are presented in

Table VIII. After the two control measurements, the oxygen content of the water was lowered to from 1.5 to 2 cc. per liter and the oxygen intake of the worms determined at this concentration for two or

TABLE VII

EFFECT OF GRADUALLY LOWERING THE OXYGEN CONTENT OVER A PERIOD OF TWO OR THREE HOURS ON THE OXYGEN CONSUMPTION OF *Planaria dorotocephala*, WATER CHANGED HOURLY*

No. of Exp.	O_2 Content at Start, Cc. per Liter	O_2 Consumed by Worms, Cc. per Hour	No. of Exp.	O_2 Content at Start, Cc. per Liter	O_2 Consumed by Worms, Cc. per Hour
15*a*	6.44	0.49	37*a*	6.50	0.53
	5.43	0.52		6.49	0.50
	5.39	0.53		4.41	0.50
	4.67	0.49		2.82	0.44
	4.13	0.50		6.68	0.53
15*b*	6.44	0.46	37*c*	6.50	0.47
	6.43	0.51		6.49	0.47
	5.39	0.49		4.41	0.44
	4.67	0.48		2.82	0.37
	4.13	0.46		6.69	0.48
10*a*	6.29	0.55	12*a*	6.48	0.49
	6.11	0.54		6.48	0.55
	5.72	0.51		4.24	0.47
	3.86	0.51		2.52	0.47
				6.39	0.57
36*b*	6.70	0.47	12*c*	6.48	0.53
	6.71	0.48		6 48	0.57
	5.10	0.52		4.24	0.51
	2.82	0.44		2.52	0.45
	2.12	0.38		6.39	0.63
36*c*	6.70	0.49	42*a*	6.29	0.34
	6.71	0.51		6.31	0.32
	5.10	0.55		3 80	0 35
	2.82	0.45		2.22	0.26
	2.12	0.38			
14*b*	5.98	0.48	42*b*	6.29	0.38
	4.64	0.43		6.31	0.46
	3.85	0.46		3.80	0.44
				2.22	0.36

* Temperature, 20° C.

three successive hours, the water being changed between successive tests. In the initial hour in the water of low oxygen tension, the usual decline in oxygen intake was noted. But during the next hours, the oxygen consumption tended to rise and even to reach the normal value, while still in the low oxygen water. This is shown in Nos. 39*a*,

39*b*, and 11*a* in Table VIII and in two others of the nine experiments performed. In the other four cases, the oxygen intake remained low during the stay in low oxygen water but rose above normal on return to normal water (Nos. 11*b*, 13*a*, *b*, and *c*). It thus appears that the oxygen intake either rises during a prolonged stay in low oxygen water and then does not rise on return to normal water, or it remains

TABLE VIII

EFFECT ON THE OXYGEN CONSUMPTION OF *Planaria dorotocephala* OF MAINTAINING A LOW OXYGEN CONTENT FOR TWO OR THREE HOURS, WITH HOURLY CHANGES, FOLLOWED BY RETURN TO NORMAL OXYGEN CONTENT*

No. of Exp.	O_2 Content at Start, Cc. per Liter	O_2 Consumed by Worms, Cc. per Hour	No. of Exp.	O_2 Content at Start, Cc. per Liter	O_2 Consumed by Worms, Cc. per Hour
39*a*	6.38	0.91	11*b*	6.68	0.60
	6.45	0.81		6.51	0.62
	1.88	0.61		1.73	0.46
	2.07	0.76		1.96	0.45
	2.21	0.81		6.53	0.71
	6.60	0.74			
39*b*	6.38	0.85	13*a*	6.37	0.43
	6.45	0.80		6.51	0.47
	1.88	0.56		1.74	0.34
	2.07	0.66		1.76	0.31
	2.21	0.71		6.36	0.56
	6.60	0.80	13*b*	6.37	0.43
11*a*	6.68	0.50		6.51	0.44
	6.51	0.53		1.74	0.32
	1.73	0.33		1.76	0.30
	1.96	0.43		6.36	0.52
	6.53	0.60	13*c*	6.37	0.50
				6.51	0.51
				1.74	0.31
				1.76	0.31
				6.36	0.64

* Total number of experiments, 9; number showing rise during low oxygen, 5; number showing rise after return to normal, 6.

at a low value while in the low oxygen water and rises above normal on return to water of normal oxygen content. In either event, a tendency to regulate to low oxygen water is manifest.

An augmentation of the normal oxygen intake following a sojourn in low oxygen water or atmospheres was noted by Gaarder (1918) for *Tenebrio* pupae (only some time after return to high oxygen atmospheres, however), by Thunberg (1905) for *Limax agrestris*,

and Lund (1921) for *Planaria agilis;* but the contrary result was observed by Lesser (1910) in the earthworm. In the latter animal, the oxygen consumption in air was lower after exposure for from three to four hours to an oxygen-free atmosphere than before such exposure.

8. *Respiration in closed flasks for several hours, the oxygen being reduced by the respiration of the animals.*—Typical data are presented in Table IX which includes about half of the experiments performed (total, 33). It is seen that the oxygen consumption proceeds at a constant rate as the oxygen is exhausted by the worms and shows no decline even at a concentration of 0.4 cc. per liter (No. 21*a*), the lowest tension attained in the experiments. Some decline, which however lies within the experimental error, appeared in part of the experiments, as 33*a* and 25*c*. It is probably to be attributed to the fact that the worms tend to become quieter during several hours without disturbance than they do in shorter periods.

It should perhaps be mentioned that the oxygen consumed by the animals is not the difference between the oxygen content at the start and at the end as given in Table IX, because these values are calculated in terms of cubic centimeters per liter. This difference would represent the actual oxygen consumed were the worms confined in flasks of exactly 1 liter content. But as the flasks used held approximately 500 cc., the actual oxygen utilization of the animals is about half the difference between the oxygen contents at beginning and end when these are stated as cubic centimeters per liter.

It thus appears that the results with low oxygen tensions vary with the method employed. The oxygen intake declines when the worms are furnished at the start with water of low oxygen content (below 3 cc. per liter) but remains constant when they exhaust the oxygen far below this value through their own respiration. A similar but less marked difference between the results with low tensions by these two methods was noted by Lund (1921) for *Planaria agilis.* The explanation which naturally occurs to one and which was also suggested by Lund, although the differences he found between the two methods were not large enough to lend much support to the explanation, is that *Planaria* is able to adjust to low oxygen water if given sufficient time. This power of adjustment appears to be much

TABLE IX

EFFECT ON THE OXYGEN CONSUMPTION OF *Planaria dorotocephala* OF CONTINUOUS RESPIRATION FOR FROM TWO TO FIVE HOURS IN AN INCLOSED FLASK, THE WORMS GRADUALLY EXHAUSTING THE OXYGEN BY THEIR OWN RESPIRATION

No. of Exp.	O_2 Content at Start, Cc. per Liter	O_2 Content at End, Cc. per Liter	Time, Hours	Total O_2 Consumed, Cc.	O_2 Consumed, Cc. per Hour
31*c*	6.44	5.22	1	0.63	0.63
	6.61	4.11	2	1.30	0.65
	6.69	3.34	3	1.74	0.58
20*a*	6.60	5.84	1	0.37	0.37
	6.11	3.06	4	1.52	0.38
33*a*	6.12	4.97	1	0.60	0.60
	6.33	4.15	2	1.15	0.57
	6.43	3.28	3	1.66	0.55
16*b*	6.45	5.68	1	0.36	0.36
	6.45	4.87	2	0.79	0.39
	6.51	4.07	3	1.24	0.41
	6.38	3.02	4	1.69	0.42
16*a*	6.45	5.58	1	0.42	0.42
	6.45	4.84	2	0.84	0.42
	6.51	4.00	3	1.33	0.44
	6.38	2.74	4	1.86	0.46
34*a*	6.40	4.60	2	0.95	0.48
	6.00	2.51	4	1.84	0.46
22*c*	6.82	4.72	2	1.08	0.54
	6.78	2.25	4	2.35	0.58
25*c*	6.85	5.76	1	0.56	0.56
	6.80	4.84	2	1.02	0.51
	6.79	2.25	5	2.36	0.47
25*a*	6.80	4.77	2	1.07	0.53
	6.79	1.96	5	2.56	0.51
22*a*	6.82	4.63	2	1.14	0.57
	6.78	1.83	4	2.56	0.64
32*b*	6.37	3.82	2	1.29	0.64
	6.32	1.51	4	2.40	0.60
21*c*	6.80	5.53	1	0.65	0.65
	6.68	4.34	2	1.18	0.59
	6.72	1.43	4½	2.76	0.61
35*b*	6.05	5.03	1	0.52	0.52
	5.85	3.90	2	0.99	0.50
	6.06	1.28	5	2.43	0.48
22*b*	6.82	4.07	2	0.69	0.69
	6.78	1.02	4	2.93	0.73
21*a*	6.80	5.45	1	0.72	0.72
	6.68	4.20	2	1.30	0.65
	6.72	0.40	4½	3.34	0.74

greater in *Planaria dorotocephala* than in *P. agilis*. That *P. dorotocephala* does possess an ability to regulate its oxygen consumption at low tensions was already indicated by the data in Table VIII, wherein it was shown that the oxygen consumption, although lowered at an initial exposure to low oxygen water, may rise even to the normal value on repeated exposure. Bearing on this point are also three experiments, presented in Table X, in which a one-hour and two-hour period in low oxygen water were compared. The decline observed in the one-hour period was not increased in the two-hour period, but the rate remained constant.

TABLE X

THREE EXPERIMENTS INDICATING ADJUSTMENT TO LOW OXYGEN WATER

No. of Exp.	O_2 Content, at Start, Cc. per Liter	O_2 Content at End, Cc. per Liter	Time, Hours	Total O_2 Consumed, Cc.	O_2 Consumed, Cc. per Hour
13*a*	6.37	5.55	1	0.43	0.43
	6.51	5.61	1	0.47	0.47
	1.74	1.09	1	0.34	0.34
	1.76	0.53	2	0.64	0.32
	6.36	5.29	1	0.56	0.56
13*b*	6.37	5.52	1	0.43	0.43
	6.51	5.63	1	0.44	0.44
	1.74	1.10	1	0.32	0.32
	1.76	0.54	2	0.61	0.30
	6.36	5.33	1	0.52	0.52
13*c*	6.37	5.40	1	0.50	0.50
	6.51	5.52	1	0.51	0.51
	1.74	1.14	1	0.31	0.31
	1.76	0.51	2	0.64	0.32
	6.36	5.13	1	0.64	0.64

All of the data strongly suggest that *Planaria dorotocephala* is able to adjust rapidly to lowered oxygen tensions and when sufficient time is allowed for such adjustment it can consume oxygen at the normal rate even in water containing less than 1 cc. of oxygen per liter.

EXPERIMENTS WITH ECHINODERMS

1. *General procedure.*—Fresh starfish and sea-urchins were collected every two or three days. As these animals live in the tidal zone, subjected to constant surf, they do not thrive under laboratory conditions. Small specimens were used suitable to the size of he

available containers. Wide-mouthed salt bottles were employed and the animals were placed in them some time before the experiments were begun. Sea water taken directly from Monterey Bay in papier-mâché buckets was employed. This is generally slightly supersaturated with oxygen. The pH is from 8.2 to 8.4. Oxygen was added from a tank when desired and low oxygen water was secured by boiling. Boiling, however, raises the pH of the sea water to about 9. This was not corrected and may have influenced the results in low oxygen water. However, as increased alkalinity accelerates oxygen consumption (cf. Anderson, 1927), any effect of the raised pH would have been contrary to the action of low oxygen water. The temperature of the water in which the animals live is about 14° C. It was not practical to maintain this temperature in the laboratory and 18° C. was selected as the lowest practical temperature. All experiments were therefore performed at 18° C. and the contents of all bottles determined at, and the oxygen calculated for this temperature.

In all cases a single animal was used in each bottle. A few tests were run on small specimens of *Patiria* to determine whether two animals together in one jar consume a different amount of oxygen from the same two animals in separate jars. No difference whatever could be found.

The majority of the experiments concerned the starfish. A lesser number was performed on the sea-urchin but this animal does not live well in the laboratory. The results on the sea-urchin will not be presented in detail but it may be said that as far as they went, they agreed precisely with the results on *Patiria*.

2. *Oxygen consumption of Patiria at increased oxygen tensions.*— Practically all of the experiments on this point are included in Table XI. It is seen that the oxygen consumption is not affected in most cases to any extent until the oxygen content is increased to from 10 to 11 cc. per liter. Above these tensions, an augmented oxygen intake is almost invariably observed. The augmentation is greater the higher the oxygen tension and is even almost proportional to the increased tension in some cases, as Nos. 13*b* and 16*b*. Where the oxygen content is increased gradually by successive steps, as in Nos. 11*a*, *b*, and *c*, and 7*a*, *b*, and *c*, the oxygen intake generally also exhibits an additional increase with each step, but not at a proportional rate.

3. *Oxygen consumption at continued high tension.*—Table XII includes all of the experiments performed on this matter. It is seen

TABLE XI

EFFECT OF INCREASING THE OXYGEN CONTENT ON THE OXYGEN CONSUMPTION OF THE STARFISH, *Patiria Miniata**

No. of Exp.	O_2 Content at Start, Cc. per Liter	O_2 Consumed by Starfish, Cc. per Hour	No. of Exp.	O_2 Content at Start, Cc. per Liter	O_2 Consumed by Starfish, Cc. per Hour
6*a*	6.18	0.50	7*c*	7.13	0.58
	6.11	0.52		6.84	0.60
	8.21	0.81		10.57	0.80
				12.27	0.94
				17.74	0.97
6*b*	6.18	0.69			
	6.11	0.78	12*b*	6.13	0.44
	8.21	0.54		5.99	0.50
				10.54	0.51
6*c*	6.18	0.71			
	6.11	0.73	12*c*	6.13	0.48
	8.21	0.59		5.99	0.57
				10.54	0.57
11*a*	6.80	0.32			
	6.82	0.43	16*a*	6.16	0.48
	9.88	0.51		6.03	0.58
	14.33	0.37		13.45	0.80
	17.86	0.60			
	6.14	0.10	16*b*	6.16	0.41
				6.03	0.52
11*b*	6.80	0.72		13.45	0.95
	6.82	0.72			
	9 88	0.81	13*a*	6.28	0.56
	14.33	1.03		19.79	1.00
	17.86	0.99			
	6.14	0.39	13*b*	6.28	0.45
				19.79	1.28
11*c*	6.80	0.74			
	6.82	0.82	5*b*	6.02	0.56
	9.88	0.79		5.97	0.62
	14.33	1.10		20.93	1.02
	17.86	1.24			
	6.14	0.59	5*c*	6.02	0.55
				5.97	0.62
7*a*	7.13	0.53		20.93	1.46
	6.84	0.47			
	10.57	0.71	5*a*	6.02	0.46
	12.27	0.78		5.97	0.50
	17.74	0.80		20.93	0.89
7*b*	7.13	0.61			
	6.84	0.69			
	10.57	0.79			
	12.27	0.83			
	17.74	0.91			

* Temperature of all experiments, 18°C.

that when the oxygen tension is held at a high value for three successive hours, the oxygen intake after an initial augmentation commonly declines and may diminish to normal or even below normal. Upon return to water of normal oxygen content, after a three-hour period at high oxygen tension, the oxygen consumption usually falls markedly below normal. This result is also in accord with that stated in the preceding paragraph. Gradual increase in oxygen content is

TABLE XII

OXYGEN CONSUMPTION OF *Patiria* AT CONTINUED HIGH TENSIONS, FOLLOWED BY RETURN TO NORMAL

No. of Exp.	O_2 Content at Start, Cc. per Liter	O_2 Consumed, Cc. per Hour	No. of Exp.	O_2 Content at Start, Cc. per Liter	O_2 Consumed, Cc. per Hour
	6.13	0.43		6.16	0.41
	5.99	0.30		6.03	0.52
12*a*.....	10.54	0.54	16*b*.....	13.45	0.95
	12.46	0.43		12.91	0.85
	11.57	0.27		12.12	0.56
				6.40	0.43
	6.13	0.44			
	5.99	0.50		6.28	0.56
12*b*.....	10.54	0.51		19.79	1.00
	12.46	0.45	13*a*.....	18.95	0.80
	11.57	0.35		18.18	0.66
				6.10	0.13
	6.13	0.48			
	5.99	0.57		6.29	0.45
12*c*.....	10.54	0.57		19.79	1.28
	12.46	0.83	13*b*.....	18.95	0.68
	11.57	0.37		18.18	0.68
				6.10	0.26
	6.16	0.48			
	6.03	0.58			
16*a*.....	13.45	0.80			
	12.91	0.48			
	12.12	0.55			
	6.40	0.33			

not accompanied by a proportional augmentation of oxygen intake, because there is a tendency for the oxygen intake to decline at continued high tensions.

4. *Oxygen consumption at low tensions.*—Practically all of the experiments concerned with this matter are presented in Table XIII. The table shows that almost any diminution in oxygen content is accompanied by a decline in oxygen intake. The lower the tension, the greater the decrease in oxygen consumption. When the oxygen con-

TABLE XIII

OXYGEN CONSUMPTION OF *Patiria* AT DECREASED TENSIONS

No. of Exp.	O_2 Content at Start, Cc. per Liter	O_2 Consumed, Cc. per Hour	No. of Exp.	O_2 Content at Start, Cc. per Liter	O_2 Consumed, Cc. per Hour
8*a*	6.04	0.67	3*b*	6.06	1.09
	5.94	0.60		6.03	0.94
	5.16	0.54		3.36	0.71
	3.12	0.36		6.14	0.93
8*b*	6.04	0.63	3*c*	6.06	0.86
	5.94	0.71		6.03	0.77
	5.16	0.56		3.36	0.56
	3.12	0.56		6.14	0.73
	5.89	0.67			
8*c*	6.04	0.68	10*a*	6.53	0.25
	5.94	0.69		6.52	0.23
	5.16	0.55		3.02	0.19
	3.12	0.38		3.18	0.17
14*b*	6.30	0.53	15*a*	7.17	0.50
	6.21	0.52		6.87	0.51
	5.24	0.42		2.68	0.38
	4.42	0.34		2.71	0.30
				3.06	0.43
				6.49	0.56
14*c*	6.30	0.45	15*b*	7.17	0.46
	6.21	0.56		6.87	0.49
	5.24	0.41		2.68	0.30
	4.42	0.35		2.71	0.27
				3.06	0.33
				6.49	0.60
2*a*	5.83	0.47	9*a*	6.35	0.60
	5.88	0.52		6.35	0.64
	4.83	0.44		2.61	0.37
				2.80	0.45
				6.29	0.65
2*b*	5.83	0.85	9*b*	6.35	0.65
	5.88	0.87		6.35	0.67
	4.83	0.82		2.61	0.45
				2.80	0.42
				6.29	0.81
2*c*	5.83	0.71	9*c*	6.35	0.70
	5.88	0.83		6.35	0.77
	4.83	0.71		2.61	0.44
				2.80	0.46
				6.29	0.82
1*a*	6.02	0.51	4*a*	6.10	0.46
	6.08	0.53		6.07	0.43
	3.87	0.40		1.56	0.23
1*b*	6.02	0.82	4*b*	6.10	0.60
	6.08	0.79		6.07	0.65
	3.87	0.46		1.56	0.22
1*c*	6.02	0.59			
	6.08	0.66			
	3.87	0.51			
3*a*	6.06	0.72			
	6.03	0.51			
	3.36	0.42			
	6.14	0.81			

tent is held at a low value for two or three hours (Nos. 15*a*, 15*b*, 9*a*, *b*, and *c*), the oxygen consumption remains about constant at a decreased value and displays some tendency to rise above normal upon return to water of normal oxygen content.

At tensions below those of the normal environment, the oxygen consumption of *Patiria* is thus dependent upon the tension. The animal exhibits no power of adjusting its oxygen intake to lowered tensions although it does possess such capacity with regard to raised tensions.

DISCUSSION

Despite numerous researches on the relation between oxygen concentration and oxygen intake in animals little understanding of the subject has been attained owing to the variability of the results. In the present investigation two forms, a planarian worm and a starfish, have been utilized, neither of which possesses, so far as known, any special respiratory mechanism nor any oxygen-carrying compounds. Both respire through the body surface. Yet one of them, the planarian, is highly independent of oxygen concentration and may respire under proper conditions at a constant rate over a range of oxygen concentration varying from 0.5 to 20 cc. per liter, while the starfish responds to almost any alteration of oxygen tension.

The two animals exhibit, however, a certain similarity in response to raised oxygen tensions. Both adjust to high tensions by a decline in oxygen intake, following an initial increase. If a relation between oxygen intake and oxygen tension is assumed to rest upon a simple physical basis, the rate of oxygen absorption would depend upon the difference between external and internal oxygen pressures. The latter would in turn depend upon the rate at which oxygen is combined to form intracellular substances concerned in the oxidation process. Any increase in external oxygen tension should result temporarily at least in augmented oxygen intake because of the steepening of the oxygen gradient. Later, however, the excess accumulation of the intracellular oxygen-utilizing substances would slow down the rate of oxygen intake. This apparently occurs in both animals during prolonged exposure to high tensions. Oxygen intake diminishes despite the high tensions, especially in the star-

fish. But in the case of *Planaria* and many other animals, large increases in external oxygen tension may have no effect whatever upon the rate of oxygen absorption. It is difficult to account for such facts through simple explanations which disregard the properties of the body surfaces. It would seem that the ability displayed by many animals of taking in only a certain amount of oxygen regardless of external tension must depend upon a capacity of the surface membranes to regulate the inward passage of the gas. This regulation appears to operate according to internal needs. It may be assumed that at ordinary oxygen tension sufficient oxygen is being absorbed to suffice for basal metabolism. More oxygen is not necessary and the body surface seems able to exclude additional intake of oxygen even at high tensions. That more oxygen can be admitted when needed is shown by the fact that the oxygen consumption of *Planaria* is more than doubled after feeding without any alteration in external tension.

The responses to low oxygen tensions offer more difficulties in the matter of interpretation. In the starfish and many other animals the oxygen intake declines as the external oxygen concentration diminishes. At first sight a simple explanation that oxygen intake depends solely upon oxygen concentration appears satisfactory and has in fact been offered by several investigators as an adequate interpretation. But it would seem if we adopt a line of reasoning similar to that given in the preceding paragraph that an internal oxygen shortage should eventually enable the animal to increase its oxygen intake after prolonged exposure to low tensions. This does occur in *Planaria* in which form the oxygen intake rises during several hours' exposure to low tensions, but no such effect was seen in the starfish. However, in the latter animal, some increase was noted on return to ordinary tensions after two or three hours' exposure to low tensions. If the animal can increase its oxygen intake over the normal at 6 to 7 cc. per liter oxygen concentration, because of a preceding oxygen shortage, why can it not do so at, say, 2 to 3 cc. content? Possibly the time at low tensions was not long enough to enable the adjustment to occur.

The behavior of *Planaria* and many other animals with respect to low oxygen tensions is still less amenable to simple physical ex-

planations. Upon initial exposure to tensions below 3 cc. per liter the oxygen intake declines, but during later exposures rises and may reach the same value as at ordinary oxygen tensions. This would seem to indicate that an internal oxygen shortage steepens the oxygen gradient with a resulting greater oxygen intake. But how can we explain the fact that, if the diminution in external oxygen tension is sufficiently gradual, the animal can continue to absorb oxygen at the normal rate even down to a concentration of 0.5 cc. per liter? It apparently becomes necessary to appeal to some obscure properties of living surfaces which enable them to absorb substances in a direction contrary to diffusion or concentration gradients. Such cases of selective absorption are well known to physiologists but so far no mechanistic explanation has been forthcoming. In fact, the secretory and excretory powers of living material have always constituted the chief stumbling-blocks in the path of physicochemical explanations of vital phenomena.

The experiments presented in this article thus seem to indicate that oxygen intake does not depend simply upon external oxygen tension even in an animal like the starfish, in which every alteration in external oxygen tension affects, initially, the oxygen consumption. Oxygen intake appears to depend primarily upon the rate of internal oxygen utilization and this in turn at any one time is determined by the history of the animal immediately preceding that time. In those animals whose oxygen intake is independent of external tension or nearly so, oxygen absorption is apparently regulated by the body surfaces, according to internal needs.

It may finally be pointed out that the differences in regard to oxygen tension displayed by the starfish and *Planaria* may represent adjustments to particular habitats. The starfish lives in an environment always supersaturated with oxygen. It consequently displays regulatory power with regard to high oxygen tensions but none or little to low tensions. *Planaria* inhabits springs in which the water contains only moderate amounts of oxygen and probably at the point of emergence from the ground rather low quantities. It is thus perhaps accustomed to adjust to low oxygen tensions. However, it is difficult to understand its equally great capacity for adaptation to very high tensions.

SUMMARY

1. The oxygen consumption of *Planaria dorotocephala*, *Patiria miniata* (starfish), and *Strongylocentrotus purpuratus* (sea-urchin) has been studied at different oxygen tensions.

2. The oxygen consumption of *Planaria* is constant between 3 and 9 cc. of oxygen per liter and in the majority of cases (85 per cent) between 3 and 17 cc. Fifteen per cent of cases showed increased oxygen content between 9 and 17 cc. per liter concentration and the majority at concentrations above 17 cc. per liter. The increase averaged 30 per cent and bore no relation to oxygen tension.

3. When *Planaria* is kept for some time at high oxygen tensions, the oxygen consumption in those cases which exhibit an initial increase at such tensions tends to fall during the exposure and may fall below normal on return to air-saturated water.

4. The oxygen consumption of *Planaria* declines during *initial* exposure to water containing less than 3 cc. of oxygen per liter, when such water is applied *directly*. The amount of decrease is greater the lower the oxygen tension.

5. When *Planaria* is kept for some time at low oxygen tensions, the oxygen consumption tends to rise and rises above normal on return to air-saturated water.

6. The oxygen consumption of Planaria remains *constant* at its value for air-saturated water when the oxygen content of the water declines *slowly*, as through the respiration of the animal, even to values as low as 0.5 cc. per liter.

7. From 4, 5, and 6 it follows that *Planaria* has the power of adjustment to low oxygen water when given sufficient time.

8. The oxygen consumption of the starfish and sea-urchin is highly dependent on oxygen content and rises and falls with the latter.

9. When the starfish is kept for some time in water of high oxygen content, the initial increase in oxygen consumption disappears and there is a marked decline in oxygen intake while in the high oxygen water and on return to air-saturated water.

10. The starfish has no capacity to adjust to low oxygen water but respires at a diminished rate while in such water. It displays

some tendency to augmented oxygen intake on return to air-saturated water after a sojourn in low oxygen water.

11. In neither the starfish nor *Planaria* does the assumption of a simple relation between external oxygen concentration and oxygen intake constitute an adequate explanation of all of the data. Both animals are able to decrease their oxygen consumption when maintained for some time at high tensions, and *Planaria* can increase its oxygen intake when kept at low tensions.

12. Rate of oxygen intake appears to depend upon rate of internal oxygen utilization, which in turn is affected by the immediately preceding history of the animal. In *Planaria*, further, the body surface probably regulates oxygen intake.

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CHANGES IN WATER CONTENT IN AMOEBA IN RELATION TO CHANGES IN ITS PROTOPLASMIC STRUCTURE[1]

(Sixteen figures)

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INTRODUCTION

LILLIE (1909) asserts that stimulation of irritable cells is dependent upon changes in permeability, and that changes in permeability are dependent upon changes in surface and interprotoplasmic films.

Verworn (1892), Chambers (1920), Bayliss (1921), Edwards (1921), Schwittalla (1921), Folger (1923), Mast (1923 and 1926), Hopkins (1926), and others account for various different physical effects produced in *Amoeba* by environmental factors, by assuming that these factors produce changes, either local or general, in "solation" or "gelation" or "fluidity"; thus implying that these produce changes in the colloid structure of the protoplasm.

Mast (1926*a*) asserts that the main cell-mass in *Amoeba* is composed of two layers, an inner more fluid layer, the "plasmasol" and an outer more rigid layer the "plasmagel." He maintains that these components differ mainly in the presence within the plasmagel of a "relatively solid framework," to which is due the rigidity of the plasmagel. This framework he asserts is probably composed of interrelated layers, i.e., it is an interprotoplasmic film structure. He describes minutely (1926*b*) the continuous transformation of the plasmasol to plasmagel, and vice versa, that forms one of the characteristic phenomena of locomotor activity in *Amoeba*, calls attention to the variation in thickness of the plasmagel with changes in external conditions, and contends that all phenomena of "solation" or "gelation" or changes in "fluidity" are correlated with local or general formation or dissolution of the film structure in the plasmagel.

[1] This article is a revision of a dissertation presented, in partial fulfilment of the requirements for the degree of Doctor of Philosophy, to the Board of University Studies of the Johns Hopkins University.

If these contentions are true, this film structure in *Amoeba*, and probably a similar structure in other cells, plays a rôle of considerable importance, and is correlated in its changes with changes in activity whether of locomotion, response to stimulus, regulation of water content, or other kindred phenomena.

Indirect evidence of this is not wanting, for every observation of change in the rigidity of the cell, however induced, may be thus interpreted. Evidence of this type, however, does not constitute proof of a relation between the state of the interprotoplasmic film structure and an external factor. It offers no opportunity for differentiation between change in rigidity involving the entire content of the cell, such as might be produced by hydration or dehydration of the entire protoplasm, and change in rigidity due to increase or decrease in the development of the interprotoplasmic film structure, which might conceivably occur with little reference to viscosity changes in the other cell constituents.

The experiments presented here were undertaken for the purpose of ascertaining whether in fact such a relation exists. They constitute an exploratory investigation into the general relation existing between the water content of *Amoeba*, as indicated by changes in volume, and the balance between the liquefying and solidifying tendencies in its cytoplasm, as shown by changes in the volumetric ratio between the more solid, film-supported plasmagel and the more liquid plasmasol.

For the convenience of the reader, the experiments on water content and on the changes in the plasmagel-plasmasol ratio are presented separately. Those dealing with the water content are presented first.

MATERIAL

The amoebae used conformed closely to the type *Amoeba proteus* (Schaeffer, 1916). They were cultured in three different media, in finger bowls: *A*, hay infusion made with half spring and half distilled water; *B* hay infusion made with modified Ringer solution[1] (Hopkins, 1926) diluted to one-thirtieth, and buffered to

[1] The modified Ringer solution was made up as follows: 0.35 gram NaCl, 0.14 gram KCl, 0.12 gram $CaCl_2$, redistilled water to make one liter of solution. In using this, 3.3 cc. of the described solution were taken to which were added 5 cc. of phosphate

pH 6.8; *C*, hay infusion made with modified Ringer solution diluted to one-sixtieth and buffered to pH 6.8. In these infusions the stems of timothy hay were used and were sterilized by fractional sterilization in the solutions used prior to adding buffer. The finger bowls contained about 200 cc. of solution each. The hydrogen-ion concentration in media *A* and *B*, which normally became alkaline with time, was maintained at the value desired by the removal when required of from 5 to 10 cc. of the media and substitution of a like amount of fresh infusion. In *C*, in which the tendency was to become acid, calcium carbonate, which had been found beneficial in unbuffered acid cultures was added from time to time until the C_H was restored to its initial value.

METHODS

Two methods were used for ascertaining the volume of *Amoeba*. They will be referred to as the "indirect" and the "direct" methods.

Indirect method.—The indirect method may be described as follows: *Amoeba* when active varies continuously in length and thickness, and tends to become spherical from time to time. When an *amoeba* is spherical the volume, of course, can be readily calculated, but the rarity of this occurrence makes such calculations of little use for experimental purposes. The diameter that an amoeba would have as a sphere can however be approximately ascertained, even if it does not actually become spherical, on the assumption that it is at all times comparable in form to an ellipsoid of rotation.

If the long axis of such an ellipsoid be varied in length, the volume remaining constant, it is obvious that as it increases the short axis must decrease and vice versa, and further that if the long axis decreases until it is equal to the short the ellipsoid becomes a sphere.

It is easily seen that given such an ellipsoid the axes of which fluctuate, but which at no time actually becomes a sphere, it is possible to secure a series of corresponding values for these axes, and, by plotting the values, to produce a graph which can be extended by extrapolation to the point at which these axes become equal to

buffer (made with KOH instead of the usual NaOH) and sufficient distilled water to make 100 cc. This is the $\frac{1}{80}$ solution, the $\frac{1}{60}$ solution is this diluted to 200 cc. of solution.

each other, and to the diameter of a sphere equal in volume to the ellipsoid.

Now, since *Amoeba* is roughly comparable to an ellipsoid of rotation, and varies greatly in length and in thickness, it is evident that, if appropriate measurements can be obtained, a corresponding series of axial values can be derived which by plotting can be used to ascertain the diameter of a sphere approximately equal in volume to the amoeba.

To obtain the required measurements of these axes of *Amoeba*, an apparatus, designated the "optical apparatus" (Fig. 1) was constructed. This consists essentially of an ordinary compound microscope which has in addition to the usual vertical objective an objective placed horizontally, and an arrangement of three prisms which reflect the image formed by the horizontal objective so that it and the image formed by the vertical objective can be viewed simultaneously by means of a single ocular. To facilitate observation with this apparatus a cell was constructed from thin slips of glass (Fig. 2).

In securing axial measurements on *Amoeba*, this apparatus was employed as follows: Using a Greenough binocular microscope and a capillary pipet, a single amoeba was selected and put into the cell together with sufficient fluid, and covered with a cover glass. The whole was placed upon the stage of the optical apparatus, and both objectives focused on the organism. There then appeared in the microscope two adjacent images which were simultaneous projections of the amoeba as viewed from above and from the side. By means of a camera lucida, outline drawings of both images were made at frequent intervals, until a series of thirty or more was secured.

Later it was found that the number could be reduced in special cases. Special care was taken to obtain in every series a great variation in length (long axis).

From the series of outline drawings thus secured the long and the short axes presented by the amoeba in each of the different forms assumed, as presented in each of the different pairs of drawings, were obtained as follows: The long axes were secured from the drawings by direct measurement to the nearest 0.5 mm. Since the two outlines of a pair were made almost simultaneously this axis was

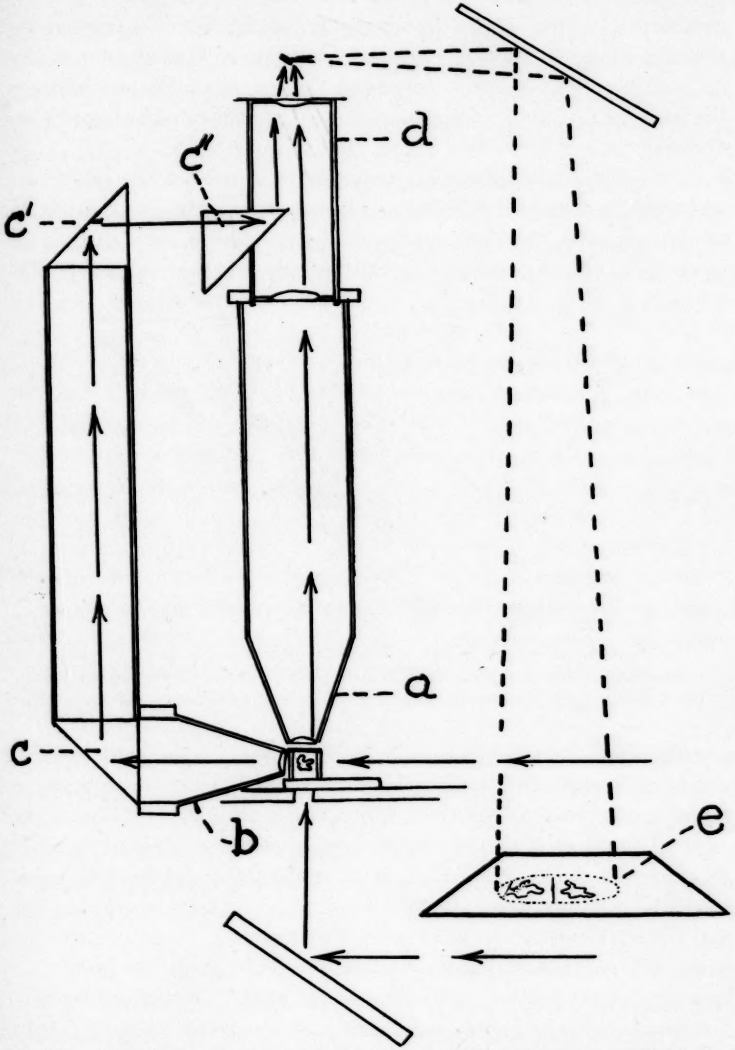

FIG. 1.—Diagram of optical apparatus used in making measurements of volume in *Amoeba: a*, vertical objective of microscope; *b*, horizontal objective; *c*, *c'*, *c''*, prisms reflecting image from horizontal objective into eyepiece; *d*, eyepiece receiving images from both vertical and horizontal objectives; *e*, images as projected by camera lucida.

common to both. The short axes were ascertained by measuring the area of each outline of each pair, dividing each of these areas by its corresponding length, computing the geometric means between the average heights and the average widths thus obtained, and then dividing each of these last values by one-quarter Pi.

This somewhat complicated method of obtaining the short axes was used to overcome the obstacles presented by the great variation in form in *Amoeba*. This variation is expressed in the drawings as irregularity of the perimeter due to laterally and vertically extended

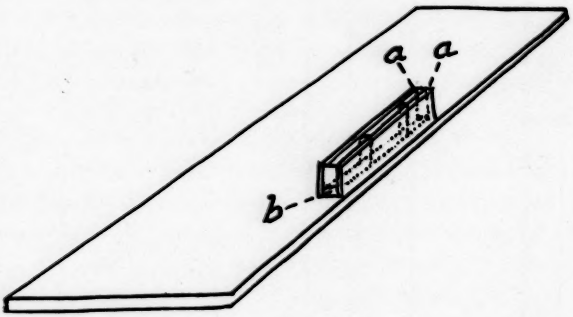

FIG. 2.—Cell used in conjunction with optical apparatus in making measurements of volume in *Amoeba: a,a*, glass sides; *b*, glass bottom; both bottom and sides are of polished glass thus permitting vision both from above and from the side.

pseudopodia. It produces such loose correlation between height, width, and length that neither height nor width, nor a mean between them, can be used as the short axis, for the basic assumption of the method requires that the linear characteristic of *Amoeba* used as this axis should vary inversely with the length, and become equal to the length only when *Amoeba* is spherical. Height or width alone will not fulfil either of these two requirements. A mean between them will partially fulfil the first but will not fulfil the last. Preliminary experimental measurements of outline drawings showed, however, that the geometrical mean between the average height and the average width of *Amoeba* does vary very nearly inversely as the long axis. This mean is equal to the long axis multiplied by one-quarter Pi, when the amoeba is spherical, and only then. It

therefore seemed that this mean divided by one-quarter Pi fulfils the requirements, and it was consequently adopted.

The areas were measured with an Ott planimeter to the nearest tenth of a square millimeter, and the lengths by scale to the nearest half millimeter.

After the axes for each pair of outlines had been measured they were plotted with the long axes as abscissas and the short as ordinates, and a smooth curve fitted to the points as described presently. This curve was then extended by extrapolation to the point where the axes would become equal, after which the volume was calculated by means of the usual formula $V = \frac{4}{3}\pi r^3$.

To test the accuracy of this method two experiments were performed, one with "plasticine" and the other with an amoeba.

Experiment 1.—A mass of plasticine was molded into a rough representation of an amoeba, laid upon a sheet of paper under a source of artificial light, and its shadow on the paper outlined with pencil. It was then turned on its long axis through 90 degrees and a second outline drawn. After this the plasticine was remolded to another form and the shadows were again projected and drawn. These processes were repeated until a series of eighteen or twenty pairs of outlines was made (Fig. 3). Axial measurements were then made from these drawings as described in the foregoing, and plotted, and then a curve was fitted to the plotted points as follows:

Trial showed that if the logarithms of the values found for the axes were plotted, instead of the actual values, the resulting graph approximated a straight line of negative slope. Hence, the relation between the axes is expressed by the formula $\log y = \log a - b \log x$, where y and x are any corresponding values for the long and short axes, respectively, and a and b are constants. The curve sought, therefore, has the formula $y = ax^{-b}$. This relation was found to hold in all graphs of measurements made on plasticine masses. The constants in the formula were evaluated from the logarithms of the axial measurements by the method of averages (Lipka, 1918, p. 126). Then, by substituting a series of values for y in the equation for the curve and solving for x a series of calculated axial values was obtained, including the value for the axes at equality. These were plotted on the graph and a curve drawn through them for com-

parison with the experimentally determined values and, finally, from the diameter of the sphere equivalent in volume to the mass, as indicated by the value calculated for the point where the axes would become equal, the volume was calculated in the usual way.

In this way, the volumes of four different masses of plasticine were calculated. Then, as a check on the calculations, the volumes

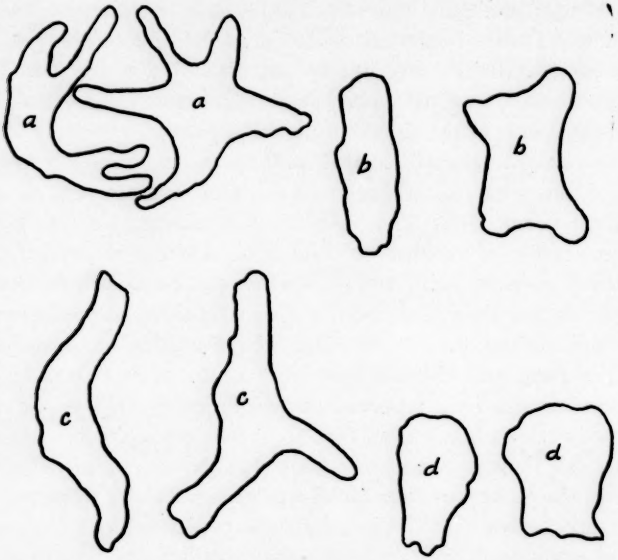

FIG. 3.—Tracings of four typical pairs of outline drawings of a plasticine model used in verifying methods of measurement; *aa*, *bb*, *cc*, *dd*, *ee* indicate the pairs; the figure to the left in each pair shows the view from above, that to the right the view from the side.

of these four masses were measured by displacement of water. The results obtained are presented in Table I and in Figure 4.

From the table it will be seen that the average percentage deviation of the calculated values from the directly measured values for volume is 5.5, indicating that if several measurements are made, and the average taken, the method is accurate to within 5.5 per cent. Since comparison between these masses and *Amoeba* might be open to question, the following experiment, using an amoeba, was performed.

Experiment 2.—An amoeba of average size was selected, and four measurements were made, each measurement being based on a series of thirty-six pairs of drawings. The results are presented in Figure 5 and in Table II.

From the table it will be seen that the four values found for the diameter are 2.97, 2.95, 3.06, and 3.00, giving an average of 2.99, with an average deviation from this value of 0.05, or 1.5 per cent, and that the corresponding values for volume are 13.18, 13.44, 14.22, and 14.14, giving an average of 13.75, with an average deviation from this value of 0.44, or 3.3 per cent. These figures represent dimensions as projected by means of the camera lucida and measured

TABLE I

MEASUREMENTS OF PLASTICINE MODELS BY THE INDIRECT METHOD COMPARED WITH MEASUREMENTS BY DISPLACEMENT

	By Indirect Method		By Displacement	
Model No.	Diameter, Cm.	Volume, Cc.	Volume, Cc.	Percentage Difference
1	3.43	21.13	21.5	1.7
2	3.83	29.28	28.0	4.4
3	3.86	30.21	32.93	9.0
4	2.87	12.43	11.56	7.0

in centimeters on the drawings. They can be transformed into terms of actual volume or diameter in cubic millimeters or millimeters by multiplying by 0.00009 or 0.44, respectively.

From the evidence thus presented as to the accuracy of the method, it is concluded that by its use the volume of an amoeba can be ascertained to within approximately 5 per cent of its actual value.

It is immediately evident, however, that the method of plotting the axes is applicable only when the volume of the amoeba remains constant throughout the period of observation, for all observations (pairs of drawings) are treated as of equal value, without reference to the time at which they were made. If changes in volume occur, modification of this method is necessary. To provide for this contingency, two other methods of plotting were devised.

In the first, which is referred to hereafter as "selective plotting,"

a series of outlines was made as before; then from these a series was selected which when arranged in order with respect to time of

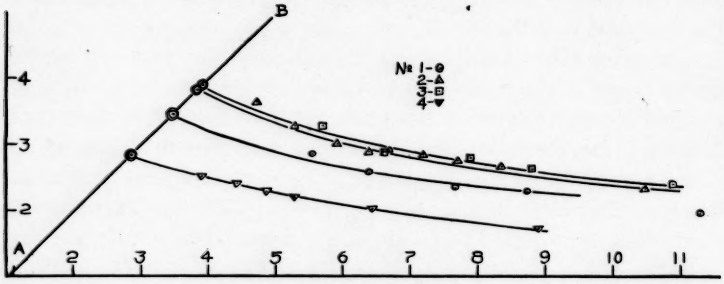

FIG. 4.—Curves obtained by plotting the axes of plasticine models molded into various amoeba-like forms. Abscissas, long axis; ordinates, short axis, of outline drawings, in centimeters: *AB*, locus of all points on the graph where the ordinates and abscissas are equal. Either co-ordinate of the intersection of the plotted curve with this line is approximately the diameter of the sphere equal in volume to the plasticine mass used.

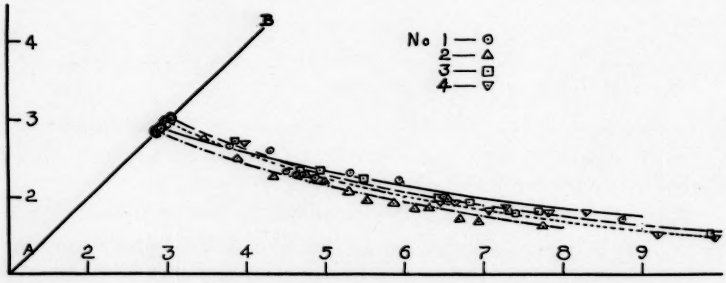

FIG. 5.—Curves obtained by plotting the axes of an amoeba. Abscissas, long axis; ordinates, short axis, of outline drawings in centimeters; *AB*, locus of all points on the graph where the ordinates and abscissas are equal. Either co-ordinate of the point of intersection of the plotted curve with this line is the approximate diameter of the amoeba when spherical, in arbitrary units, i.e., in centimeters as measured on the outline drawings. This diameter may be converted to the actual diameter of the amoeba in millimeters by multiplying by 0.044 and the volume calculated by means of the ordinary formula for the sphere.

making during the observation period were also arranged so that each pair had a shorter long axis than the preceding pair. From

this series the data for the axes were secured as described in the foregoing and plotted, and a curve passed as before. This method of plotting is satisfactory for ascertaining the volume at the expiration of the period of observation, but it gives little information as to the rate of change in volume, because the time intervals between the observations do not appear in the graph.

The second method, called hereafter "group plotting," overcomes this obstacle. In this a series of outlines is made as before,

TABLE II

RESULTS OF FOUR MEASUREMENTS OF ONE AMOEBA BY THE INDIRECT METHOD*

Diameter	Volume	Deviation
2.93	13.18	.57
2.95	13.44	.31
3.06	14.22	.47
3.00	14.14	.39
Average	13.75	.44

* The volume is given in arbitrary units. The actual volume in cubic millimeters is obtained by multiplying by 0.00009.

and arranged in order in reference to the time each was made. Then, on the basis of the similarity of *Amoeba* to an ellipsoid of rotation, the volume of the amoeba observed is calculated from each set of outlines of the series, on the assumption that it is equal, approximately, to that of an ellipsoid with axes equal to those of the amoeba as recorded in the outlines of the series. After this, the averages obtained from successive groups of three or five of these volumes are plotted against time, and a curve is obtained that shows approximately the rate of change in the volume of the amoeba over the period of observation. In graphs given here, such curves are replotted in terms of percentage change (loss or gain) of the initial volume, so as to facilitate comparison. Where change from the ordinary method of plotting measurements by the indirect method was made it is indicated as noted in the foregoing.

Direct method.—In the "direct method" use was made of an apparatus consisting of a pyrex glass pipet having a capillary constriction, and an appliance for producing suction whereby an amoeba introduced into the pipet can be drawn into the constricted portion,

retained there during observation, and ejected without injury (Fig. 6). This apparatus will be referred to as the volumescope. It is used in measuring *Amoeba* as follows.

An amoeba together with sufficient fluid is placed in the mouth of the volumescope by means of a fine pipet. Then, by slightly unscrewing the cap (Fig. 6, *c*) it is sucked into the capillary part of the tube (Fig. 6, *b*). After this the volumescope is placed upon the stage of a microscope, and the amoeba is brought, by manipulation of the cap, to a predetermined part of the tube and retained there while a sketch is made of it by means of a camera lucida. Finally,

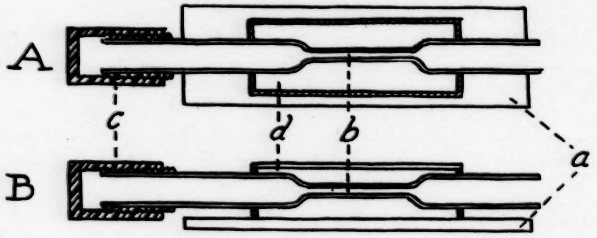

FIG. 6.—Volumescope used in ascertaining the volume of *Amoeba*. *A*, viewed from above; *B*, viewed from the side; *a*, glass microscope slide; *b*, pipet tube with capillary constriction into which the amoeba can be drawn by suction; *c*, screw cap by manipulation of which the organism is drawn into the capillary; *d*, glass-covered casing containing oil to reduce refraction and make clear observation of the amoeba while in the capillary possible.

it is ejected from the capillary portion of the tube by further manipulation of the cap. If several measurements are required, as is usual, the amoeba is left in the mouth of the volumescope until normal shape and activity are restored, after which it is again drawn into the capillary and sketched. This operation may be repeated, without injury to the amoeba, as often as desired, provided sufficient time is allowed to elapse between successive repetitions.

Since the diameter of the lumen of the capillary part of the volumescope is less than the normal diameter of the amoeba, traction into this part results in change in its form to conform to that of the lumen of the tube, i.e., the amoeba is forced to assume a cylindrical form. When in this form the volume is easily ascertained, as it is equal to $L\pi r^2$, in which L is the length of the amoeba, and r

the radius of the capillary, dimensions readily obtained from the sketches. Obviously this formula is applicable only if the ends of the amoeba take the form of parallel planes. In practice these ends are not planes but are variously curved surfaces; the measurement of length was therefore not made between the actual ends as sketched, but between points fixed by estimating the position that the ends would occupy if plane.

Change in volume is of course directly proportional to the observed change in length, provided that the same volumescope is used throughout. Only observations made with the same instrument are compared in this article.

This method necessarily involves stimulation of considerable severity, and the results presented in the following section show that such stimulation produces marked changes in volume. They, however, also show that if measurements are made in groups of five, and the interval between these groups is from ten to fifteen minutes, errors resulting from the stimulation involved in the act of measuring may be neglected.

CHANGES IN TOTAL VOLUME

THE EFFECT OF MECHANICAL STIMULATION

Lillie (1923), Lund and Logan (1925), and others assert that mechanical stimulation of cells results in alterations of permeability, and hence changes in volume. It seemed desirable to ascertain to what extent such changes in volume occur in *Amoeba*, especially so since in the application of the "direct method" of measurement the amoeba is strongly stimulated mechanically. Accordingly three experiments were performed in each of which an amoeba was subjected to a different frequency of mechanical stimulation.

Experiment 1.—An amoeba was taken from the culture and put into the volumescope, in culture fluid, and fifty measurements of its length, maximum and estimated, made at intervals of from two to three minutes. These two series of lengths were then averaged by tens and the resultant values compared with the average of the first ten in each series, and the differences expressed as percentage loss or gain in respect to this average, plotted against time. The maximum lengths served as a control for the error involved in esti-

mation. This was repeated with a second amoeba; the results are presented in Figure 7.

It will be seen from this figure that the first amoeba lost 8 per cent in length (proportional directly to volume) in ninety minutes, and the second 7 per cent in eighty minutes.

Experiment 2.—Ten measurements of an amoeba were made with the volumescope. Then it was transferred to a watch glass and

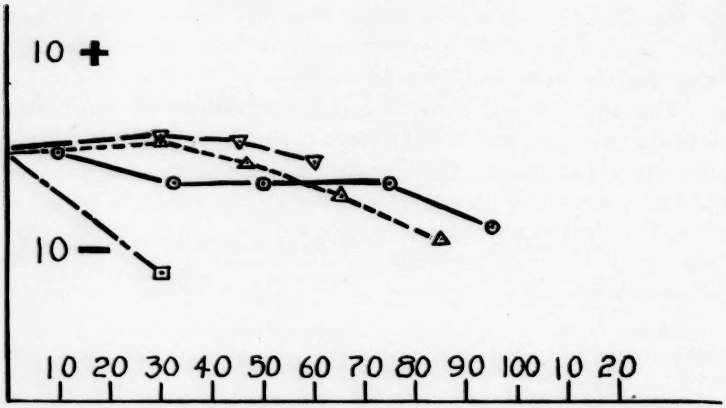

FIG. 7.—Graph showing changes in volume of *Amoeba* subjected to mechanical stimulation of various frequencies. Abscissas, period of stimulation in minutes; ordinates, losses or gains in volume as percentages of original volume; square symbols, points obtained with high-frequency stimulation; circles and triangles, points obtained with moderate-frequency stimulation; inverted triangles, points obtained with low-frequency stimulation.

agitated for thirty minutes with a glass rod. After this ten more measurements were made; the results as in the previous experiment are presented in Figure 7.

From the figure it will be seen that there was a decrease in volume of 12 per cent in thirty minutes, while in the preceding experiment there was a decrease of only 8 per cent in ninety minutes, and it is therefore concluded that the loss in volume varies directly with the frequency of the stimulation.

Experiment 3.—An amoeba was measured ten times with intervals of two or three minutes, as in the first experiment. Then the

amoeba was allowed to rest for fifteen minutes, after which the process was repeated until forty measurements had been made. The results are also presented in Figure 7.

It seems evident from these results that little if any change in volume occurred, or that if such change did occur the rest period allowed was sufficient for recovery.

The accuracy of the measurements is indicated by the fact that the average deviation of the forty measurements of the third experiment is about 2 per cent of the mean. It is therefore concluded that the method can be considered accurate to within 5 per cent or less. For this reason, and for the reason that measurements can be made with considerable ease and frequency, it is further concluded that it is preferable to the indirect method previously described in the first section, except perhaps where it is important to avoid all mechanical stimulation.

THE EFFECT OF HYPERTONIC SOLUTIONS

1. Glycerol: indirect method.—In using the indirect method the amoeba to be measured must be kept under observation from one to three hours. It was therefore necessary, first, to ascertain the maximum concentration to which *Amoeba* can be subjected for this length of time without injury. This was done as follows:

Ten amoebae were put into each of three watch glasses containing respectively 0.1 M, 0.2 M, and 0.3 M glycerol solution made up in culture fluid,[1] and observed at intervals over a period of three hours. It was found that the amoebae in 0.1 M and 0.2 M glycerol ceased locomotion immediately, and that at the end of three hours four in 0.3 M had disintegrated, and all the rest had become rounded and motionless. After these observations were made, all the amoebae were transferred to culture fluid. Those taken from the 0.1 M and 0.2 M solutions recovered completely in twelve hours. Those from the 0.3 M solution never recovered. The maximum concentration that can be used with this method is therefore somewhat lower than 0.3 M. The following concentrations were selected: 0.275 M, 0.2 M, and 0.1 M.

The effect on *Amoeba* of these concentrations was ascertained as

[1] Cultures used maintained by method A.

follows: An amoeba was selected from culture and two measurements (the result of calculation based on a series of sets of outlines is referred to as a measurement and a single set of two outlines is termed an observation) were made of it in culture fluid. Then 0.275 M glycerol solution was introduced into the cell, replacing the culture fluid, and a third measurement made. The observations supporting this measurement were thirty in all and covered a period of approximately two hours. After this the amoeba was put into a watch glass, washed with five changes of culture fluid, and left in culture fluid with food for twenty-four hours, after which it was again measured twice in culture fluid. It was now put into culture fluid once more and a few observations made; then the culture fluid was replaced by 0.2 M glycerol solutions, and the measurement completed, after which it was again transferred to culture fluid and washed. During the recovery period, however, it divided, preventing further measurements of this individual. A second amoeba was therefore selected, measured four times in culture fluid, and then in a 0.1 M glycerol solution.

Selective plotting was used for all observations constituting the measurements in glycerol, and the data for the amoeba in 0.1 M glycerol were also treated by group plotting. No measurement was based on less than thirty observations.

The results obtained are presented in Figures 8, 9, and 10 and in Table III.

From Figure 8 it will be seen that the curves derived from the observations in culture fluid formed two compact groups, and that in each group all the curves intersect the line AB at nearly the same point, indicating that there was but little variation in the results of the observations. It will also be seen that the curves obtained from the observations in the glycerol solutions all intersect the line AB nearer to the origin than the curves obtained from the observations in culture fluid, proving that in these solutions the amoebae decreased in volume. It will be seen from Figure 9 that this decrease varied directly with the concentration of the solutions. The absolute changes in volume can be seen in Table III, and from this it will be noted that the amoebae in 0.275 M and 0.2 M, and in 0.1 M glycerol lost 49, 29, and 19 per cent in volume respectively.

In addition to these quantitative results, it is to be noted (Fig. 8) that in the measurement in 0.2 M and in 0.1 M glycerol solution the curve obtained has a sharp break indicating a sudden drop in volume. However, in the 0.2 M solution this decrease occurred immediately after the introduction of the glycerol (the points to the right of the break are determined by calculation from the observa-

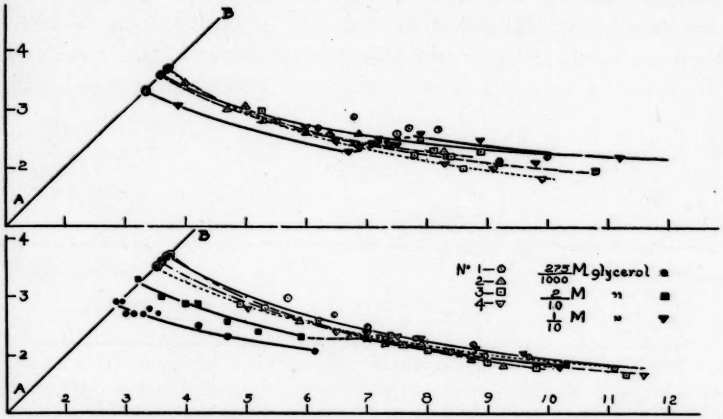

FIG. 8.—Curves plotted from axial measurements of two amoebae immersed in culture fluid, contrasted with curves obtained by plotting like measurements of the same amoebae in glycerol solutions of 0.1, 0.2, and 0.275 M concentrations. Abscissas, long axis; ordinates, short axis, as obtained from measurements of camera lucida drawings; *AB*, locus of all points on the graph where ordinates and abscissas are equal. Either co-ordinate of the point of intersection of each curve with this line is approximately the diameter of the amoeba when spherical in arbitrary units, i.e., centimeters as measured on the outline drawings. The actual diameter in millimeters can be obtained by multiplying by 0.044. Open symbols, points obtained from measurements in culture fluid; solid symbols, points obtained from measurements in glycerol solutions; solid fine line, first measurement; dot and dashed line, second measurement; dashed line, third measurement; dotted line, fourth measurement (all four in culture fluid); solid heavy lines, measurements in glycerol solutions. Note that all curves obtained for the amoebae in glycerol intersect the line *AB* nearer to the origin than any curve obtained from the same amoeba in culture fluid, proving that the amoebae decreased in volume in the glycerol solutions.

tions in culture fluid and those to the left from the observations in glycerol) while in the 0.1 M solution it did not occur until nearly two hours after the organism had been put into the glycerol, as is

shown in Figure 9. This would seem to indicate that in the more dilute solution some regulative activity in the organism prevented an immediate decrease in its volume.

2. Lactose: indirect method.—The effect of lactose was ascertained as follows: An amoeba was put into $\frac{1}{3}\frac{1}{0}$ modified Ringer solution at pH 6.8 without food,[1] left for three hours, and then transferred to the cell and measured. After this it was returned to the Ringer solution, the cell filled with 0.15 M lactose solution, made up in $\frac{1}{3}\frac{1}{0}$ Ringer solution, the amoeba replaced, and again measured. The amoeba died 114 minutes after the measurement was begun.

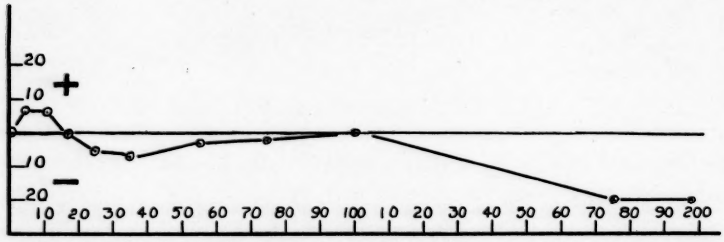

FIG. 9.—Graph showing approximate rate of change in volume in an amoeba immersed in 0.1 M glycerol solution. Abscissas, time in minutes from moment of immersion; ordinates, percentage loss or gain in volume. Note that the small fluctuations in the first part of the curve are within the experimental error, no definite change being shown until one hundred minutes have elapsed.

This was thought to be unusual, so a second amoeba was selected and subjected to the same treatment. It was in the solution 170 minutes, after which it was transferred to modified Ringer solution and found to be uninjured. The test was then repeated with another amoeba, using the direct method. Five measurements were made in $\frac{1}{3}\frac{1}{0}$ Ringer solution, and then four sets of five in 0.15 M lactose solution, one thirty, one sixty, one one hundred and five, and one one hundred and eighty minutes after the amoeba had been introduced. The results of the observations on the two amoebae measured by the indirect method were treated by group plotting Figure 11.

From this figure it will be seen that the three amoebae decreased in volume 45, 48, and 55 per cent, respectively, and that the second amoeba recovered completely on transfer to the Ringer solution.

[1] Cultures used maintained by method B.

The first as noted above died, and the third was not measured after the experiment. It will also be seen that the curves obtained are all convex to the axis of abscissas, indicating that in every test the rate

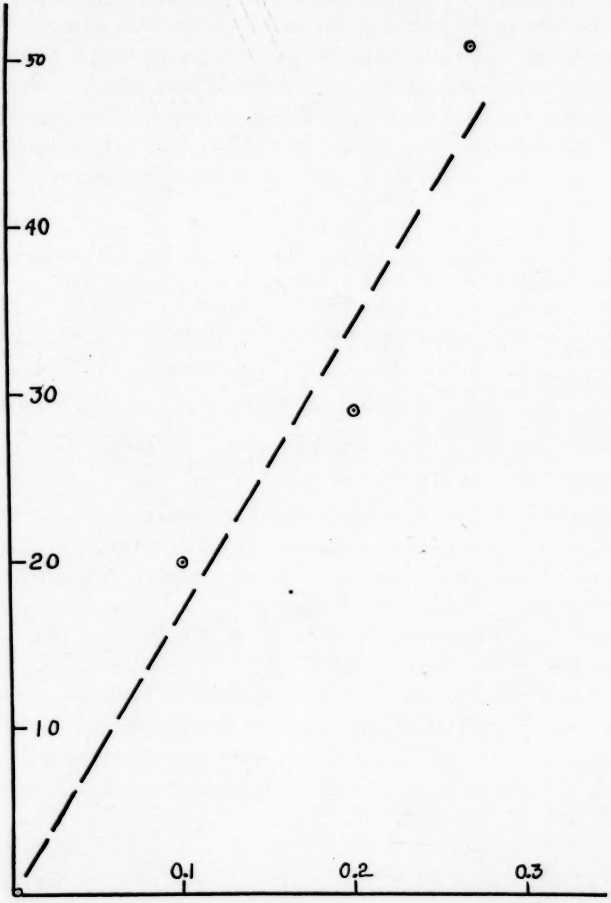

FIG. 10.—Graph showing relation between the volume of *Amoeba* and the concentration of glycerol in the surrounding medium after approximately two hours immersion at each concentration. Abscissas, concentration of glycerol in mols per liter; ordinates, percentage loss in volume.

of decrease in volume decreased with time. It is also to be noted that the percentage of decrease in volume in 0.15 M lactose is approximately the same as that in 0.275 M glycerol, indicating that the lactose solution is more effective (penetration of glycerol?).

3. Urea.—In ascertaining the effect of urea the procedure was essentially the same as that of the preceding experiment. A 0.15 M solution of urea made up in $\frac{1}{30}$ modified Ringer solution was used. One amoeba was measured by the indirect method, and four by the direct. The results obtained by the indirect method were plotted by groups. The results obtained by both methods are presented in Figure 12.

TABLE III

CHANGES IN VOLUME IN AMOEBAE IMMERSED IN 0.275, 0.2, AND 0.1 M GLYCEROL SOLUTIONS ASCERTAINED BY USE OF THE INDIRECT METHOD*

No. Vol. in Culture Fluid	Vol. in 0.275 M Glycerol	Percentage Decrease	Vol. in 0.2 M Glycerol	Percentage Decrease	Vol. in 0.1 M Glycerol	Percentage Decrease
1. 25.5	12.36	49	18.15	29	.....	.....
2. 25.1	.....	.....	.....	.....	20.3	19

* The time the amoebae were in the solution was in all cases approximately two hours. The actual volumes in cubic millimeters can be obtained from the above figures, which are in arbitrary units, by multiplying by 0.00009.

It will be seen from this figure that the amoeba measured by the indirect method decreased 40 per cent in volume, and that the four measured by the direct method decreased respectively 32, 23, 19, and 35 per cent. The period of observation was approximately two hours except in the case of the amoeba which lost 19 per cent. This amoeba, however, died after fifty minutes in the solution. It will also be noted from the figure, that the curves at first are somewhat convex toward the axis of abscissas, then nearly parallel to it, and finally concave, indicating that after a short period during which the amoebae decreased fairly rapidly in volume, the rate of decrease lessened only to become a little later more accelerated. Two hours after the experiment was concluded, all amoebae were dead, indicating that the acceleration in the decrease in volume continued until death resulted.

It is evident that there is some discrepancy between the curve obtained for the amoeba measured by the indirect method and those secured from the direct measurements, which among themselves are

very consistent. This is accounted for in part by the fact that the amoebae measured by the two methods were taken from different

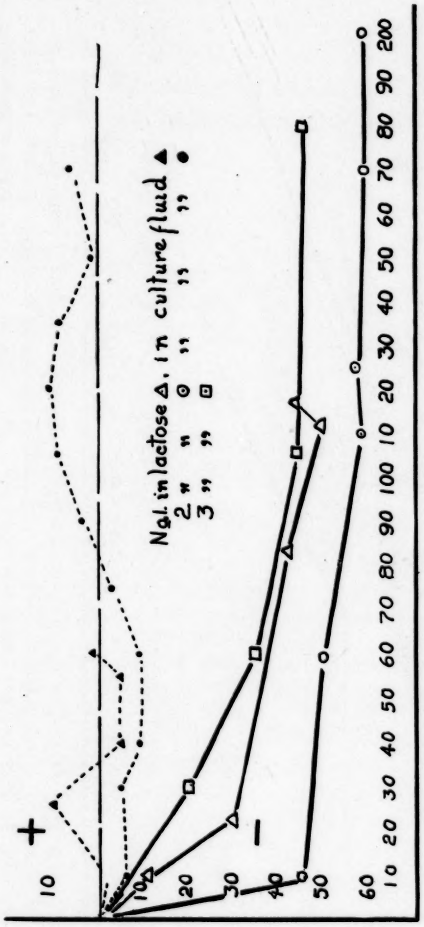

FIG. 11.—Graph showing the rate of change in volume of three amoebae immersed in 0.15 M lactose made up in modified Ringer solution. Abscissas, time in minutes from moment of immersion; ordinates, percentage loss or gain in volume; solid lines, curves for three amoebae in lactose solution; dotted lines, curves obtained from two of these amoebae in $\frac{1}{3}$ modified Ringer solution previous to immersion in the lactose. The fluctuations represented in these give some indication of the experimental error. Note that in all amoebae the rate of loss in volume decreased with time. The last two points in the dotted curve for amoeba No. 2 were obtained after recovery from immersion in lactose.

cultures, and in part by the difference in the accuracy of the methods. Moreover, the discrepancy is but quantitative and does not affect any general conclusion based on the trend of all of the curves.

By comparing Figures 11 and 12, it will be seen that the rate of decrease in volume in urea follows a different course from that in lactose.

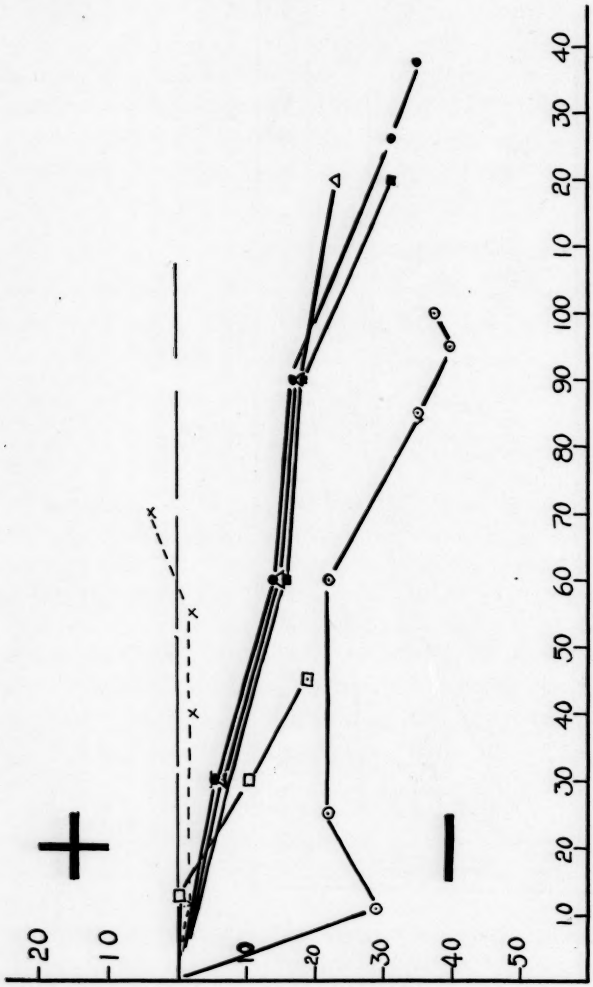

FIG. 12.—Graph showing the rate of change in volume in five amoebae immersed in 0.15 M urea made up in $\frac{1}{30}$ modified Ringer solution. Abscissas, time in minutes from immersion; ordinates, percentage loss or gain in volume; solid lines, curves for amoebae in urea solution; dotted line, curve obtained from one amoeba immersed in $\frac{1}{30}$ Ringer solution. The fluctuation in this curve shows the experimental error. The curve with circles was obtained from measurements by the indirect method, the rest by the direct method, and the amoeba used in the former was not from the same culture as those in the latter. All curves show first a decrease in rate of loss in volume and later an increase in this rate.

HYDROGEN-ION CONCENTRATION

Twenty-four amoebae[1] were washed in modified Ringer solution and put into modified Ringer solution at pH 7.0, left for three hours, and then measured by means of the volumescope, after which each one was put separately into a 2-cc. glass dish containing modified Ringer solution. In six of the dishes the C_H of the solution was pH 6.0, in six, pH 6.6, in six pH 7.0, and in six pH 8.0. These dishes

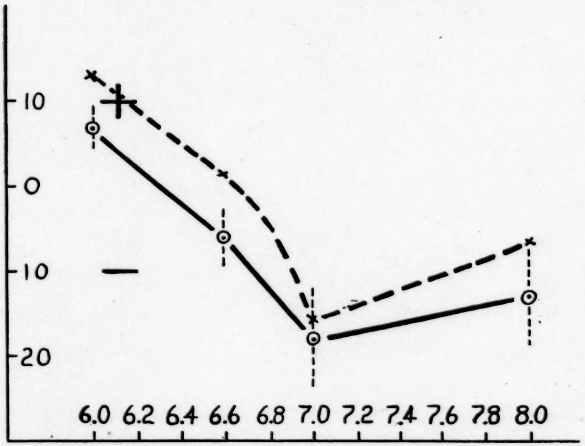

FIG. 13.—Curves showing the relation between volume in *Amoeba* and the hydrogen-ion concentration of the surrounding medium. These are based on the measurement of twenty-four amoebae immersed in $\frac{1}{6}$ Ringer solution at different hydrogen-ion concentrations. Abscissas, pH; ordinates, percentage loss or gain in volume; dotted lines through points show the extent of the probable error for each point.

were arranged in four equal groups in which the hydrogen-ion concentration of the contained solutions was respectively pH 6.0, 6.6, 7.0, and 8.0. Each group was then put into a 10-cc. pyrex glass dish containing enough modified Ringer solution of the same hydrogen-ion concentration as that in the 2-cc. dishes to completely immerse these small dishes. The amoebae were left in the small dishes for three hours and then again measured. The results obtained are presented in Figure 13 and in Table IV.

[1] Cultures used maintained by method C.

From the table it will be seen that there was an increase in volume in all the amoebae in the modified Ringer solution at pH 6.0,

TABLE IV

CHANGES IN VOLUME IN AMOEBAE IMMERSED IN MODIFIED RINGER SOLUTIONS AT DIFFERENT HYDROGEN-ION CONCENTRATIONS*

Amoeba	Initial Volume	Vol. after 3 Hours in Ringer Solution at pH 7.0	Percentage Change in Volume	Vol. after a Further 3 Hours in Ringer Solution at pH 6.0	Percentage Change in Volume
1	8.0	7.5	− 6	8.3	+10
2	8.7	8.8	0	8.9	0
3	8.0	8.0	0	8.8	+10
4	10.4	8.8	−15	9.6	+ 9
5	6.3	5.6	−11	5.8	+ 4
6	7.3	lost			
Average			− 6		+ 7

Amoeba	Initial Volume	Vol. after 3 Hours in Ringer Solution at pH 7.0	Percentage Change in Volume	Vol. after a Further 3 Hours in Ringer Solution at pH 6.6	Percentage Change in Volume
7	6.8	6.2	− 9	5.7	− 8
8	8.6	8.0	− 2	7.4	− 8
9	8.7	8.0	− 8	7.7	− 5
10	9.2	9.0	− 2	9.0	0
11	9.1	7.4	−17	lost	
Average			− 7		− 6

Amoeba	Initial Volume	Vol. after 3 Hours in Ringer Solution at pH 7.0	Percentage Change in Volume	Vol. after a Further 3 Hours in Ringer Solution at pH 7.0	Percentage Change in Volume
12	9.2	8.0	−13	7.3	− 9
13	6.3	6.5	+ 3	5.5	−16
14	7.5	7.4	0	6.1	−18
15	6.3	6.2	0	4.3	−33
16	9.0	8.5	− 6	7.5	−11
17	6.8	lost			
Average			− 3.2		−18

* Each figure is the average of five readings. To obtain actual volumes multiply figures by 0.0003. This equals the volume in cubic millimeters.

and a decrease in volume in the solutions at pH 6.6, 7.0, and 8.0. This appears certain in spite of considerable variation in the extent

TABLE IV—*Continued*

Amoeba	Initial Volume	Volume after 3 Hours in Ringer Solution at pH 7.0	Percentage Change in Volume	Vol. after a Further 3 Hours in Ringer Solution at pH 8.0	Percentage Change in Volume
18	6.5	6.9	+ 6	4.2	−30
19	7.0	6.7	−11	6.1	−10
20	8.4	8.3	0	7.6	−10
21	6.2	5.9	− 5	5.2	−12
22	7.4	6.1	−18	5.9	− 4
23	10.0	8.7	−13	8.0	− 6
24	9.0	lost	.....	.....	.....
Average..	.....	.....	− 6.6	.....	−13

of the changes in volume in individual amoebae. The curve (solid line) in Figure 13 indicates that under the conditions which obtained no change in volume occurs in modified Ringer solution at about pH 6.4, that in solutions having a higher hydrogen-ion concentration than this the volume increases, and that in those having a lower concentration it decreases.

This result does not indicate that transfer of *Amoeba* from culture fluid at pH 6.8 to modified Ringer solution at the same hydrogen-ion concentration, i.e., to the same solution without food, results in a decrease in volume, as such conclusion neglects the first transfer to modified Ringer solution at pH 7.0, which resulted in a loss in volume. To evaluate more correctly the effect on *Amoeba* of transfer from culture fluid to modified Ringer solution at the hydrogen-ion concentrations used, allowance must be made for this decrease. If this is done, by adding back the decrease in volume resulting from this initial transfer and assuming that the direct transfer would result in the same percentage changes as did the final transfers actually made, i.e, from Ringer solution at pH 7.0 to the various solutions, a curve is obtained, Figure 13 (dotted line), that indicates that if the initial transfer had been omitted the volume would have been found to be unchanged at pH 6.8 (hydrogen-ion concentration of culture) to increase in solutions more acid than this, and to decrease in those more alkaline. The greatest decrease, however, would still be found at pH 7.0. This would indicate that *Amoeba* cultured in acid solutions transferred to solutions more acid than that in which it is grown tends to gain in volume, and if trans-

ferred to solutions more alkaline to decrease in volume, but that it decreases most rapidly in a neutral solution.

Hopkins (1927) asserts that the water content of *Amoeba* is dependent upon the hydrogen-ion concentration, and (1926) that the water content is greatest at neutrality. It is evident that the foregoing confirms the first contention but disproves the last.

Assuming that the changes in volume observed are due to changes in water content the results thus far presented may be summarized as follows:

1. Mechanical stimulation of *Amoeba* results in a decrease in water content. Low frequency is less effective than high.

2. The water content of *Amoeba* decreases in hypertonic solutions of glycerol, lactose, and urea. Of these the most effective is lactose. In glycerol the percentage of decrease in volume varies directly with the concentration used. In lactose the rate of water loss decreases with time. In urea the rate of loss at first decreases and then increases. This increase probably continues until death results.

3. The water content of *Amoeba* is dependent upon the hydrogen-ion concentration of the medium. Evidence is presented tending to show that if *Amoeba* is transferred from acid culture to more acid solutions it increases in volume, and that it decreases in volume if transferred to more alkaline solutions, but that it decreases in volume most rapidly if transferred to a neutral solution.

CHANGES IN THE VOLUMETRIC RATIO BETWEEN THE PLASMAGEL AND THE PLASMASOL

It is well known that the plasmagel in *Amoeba* changes freely into plasmasol and vice versa, and that, as previously stated, these changes form one of the characteristic phenomena of locomotion (Mast, 1926*b*). Nothing is definitely known as to the cause of this transformation, but it has been surmised that changes in the water content, and in the hydrogen-ion concentration within the cell, are involved. Changes in the ratio between the plasmagel and the plasmasol have been observed, and it has been assumed that these changes depend upon environmental conditions. The experiments presented in the following pages have an important bearing upon this problem. They constitute an attempt to correlate measured changes

in the volumetric ratio between the plasmagel and the plasmasol, with definite environmental changes, and thus to ascertain some of the factors involved in formation and dissolution of the plasmagel and the plasmasol.

Two methods, A and B, were used to ascertain the volumetric ratio between the plasmagel and the plasmasol, referred to hereafter as the gel-sol ratio.

In method A the volume of the entire organism, and that of the plasmasol, was ascertained by the indirect method, group plotting being used in handling the results, and from the two curves produced, one showing the changes in the total volume and the other the changes in the volume of the plasmasol throughout the period of observation, the corresponding changes in the gel-sol ratio were readily calculated.

In method B an amoeba was put into the volumescope, placed under a microscope, and drawings made of typical sections of the organism, showing the limits of the plasmasol and the entire organism. From these drawings a series of values was obtained for the average diameter of the plasmasol and the entire amoeba, while it was in the volumescope. The squares of these values multiplied by the length of the amoeba were taken as roughly proportional to the volumes of the plasmasol and the whole amoeba, respectively, and from them the gel-sol ratio was obtained. By computing this ratio at intervals and plotting against time, a curve showing the changes in it during a period of observation is readily obtained.

By means of these two methods records were obtained of the changes in the gel-sol ratio produced by lactose, urea, and hydrogen-ion concentrations identical with those used in the previous experiments, and observations were made of the effect upon it of mechanical stimulation.

Lactose.—At the time measurement was made on the effect of lactose on the total volume in *Amoeba*, described in the preceding section under indirect method, measurements were also made of the volume of the plasmagel. From the results obtained in these two sets of measurements the changes in the gel-sol ratio during the period of observation were computed by method A. The results obtained are presented in Figure 14.

From this figure it will be seen that in one of the amoebae the gel-sol ratio increased, in fifteen minutes, from its initial value of 6.4 to a maximum of 18.0, and then decreased slowly until at the close of the experiment it reached approximately its original value; and that in the other amoeba the gel-sol ratio from an initial value of 7.4 also increased to a maximum and then decreased, but that the time taken to reach the maximum was much greater, thirty minutes, and the maximum value was only 9.3. This difference is probably connected with the fact that the first amoeba died shortly after the measurements of the plasmasol were completed. This, taken in connection with the large fluctuation in the gel-sol ratio, indicating an extreme gelation of the protoplasm, is thought to indicate an abnormal physiological state, especially since death occurred after a loss of only 49 per cent of total volume, as compared to a loss of 59 per cent in the second amoeba which suffered no injury (other than the usual rounding up that occurs in these strongly hypertonic solutions). It seems better, therefore, to consider the changes in the ratio in the second amoeba as presenting the more normal picture. By comparing these changes with the changes in total volume occurring simultaneously with them, it will be noted that during the early part of the experiment, while there was a great decrease in volume, indicating a correspondingly great decrease in water content, the gel-sol ratio increased slightly, but that during the later part of the experiment when there was but slight change in the total volume the gel-sol ratio gradually returned toward its original value.

This indicates that during the first part of the experiment water was lost from the plasmasol more rapidly than from the plasmagel, even though the plasmagel was closer to the hypertonic solution, and leads to the conclusion that the plasmagel has a greater affinity for water than the plasmasol, and acts as a semipermeable membrane between the plasmasol and the exterior. The return of the gel-sol ratio toward its initial value apparently indicates that as osmotic equilibrium is reached in respect to the exterior medium, there is a redistribution of the water content in the cell.

Urea.—The changes in the gel-sol ratio produced by immersion in urea were ascertained from measurements on two amoebae, one

measured by method A and the other by method B. The measurements by method A were secured at the same time as the measurements of the effect of 0.15 M urea on the total volume presented in the preceding section. The measurements by method B were made as described in the foregoing, in groups of five, the averages of these groups being taken. One group of five measurements was made before the immersion of the amoeba in the urea solution, and then

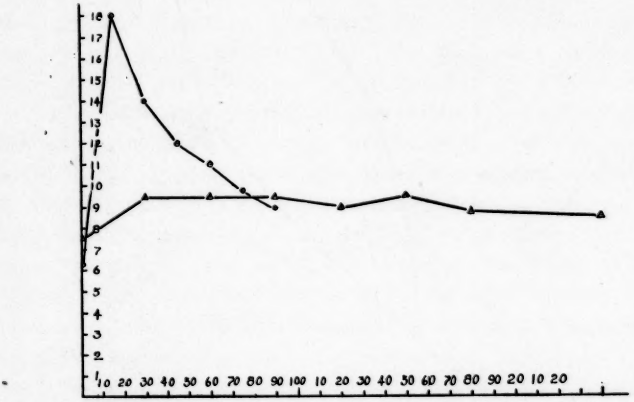

FIG. 14.—Graph showing changes in the volumetric ratio between plasmagel and plasmasol in two amoebae immersed in 0.15 M lactose made up in $\frac{1}{10}$ modified Ringer solution. Abscissas, time from immersion in minutes; ordinates, plasmagel-plasmasol ratio. The amoeba showing the very large increase and recovery of ratio died in 114 minutes after immersion. Compare these curves with Curves 1 and 2 in Figure 11, showing the changes in volume in these two amoebae during the same period.

seven more groups at five, fifteen, thirty, forty-five, sixty, seventy-five, and ninety minutes after immersion. The results obtained are presented in Figure 15.

From this figure it will be seen, on comparing the curve obtained by method A with that obtained by method B, that one indicates that there was a rise in the gel-sol ratio to a maximum during the first ten minutes, and the other does not. The fact, however, that the curve obtained by method A indicates that there was no rise does not demonstrate that a rise did not occur for no measurements were made during this time. Moreover, there is some evidence

which indicates that it actually does occur. This is presented in the following paragraph.

In lactose it has been demonstrated that there occurs a rise of the gel-sol ratio to a maximum, that this rise to a maximum is

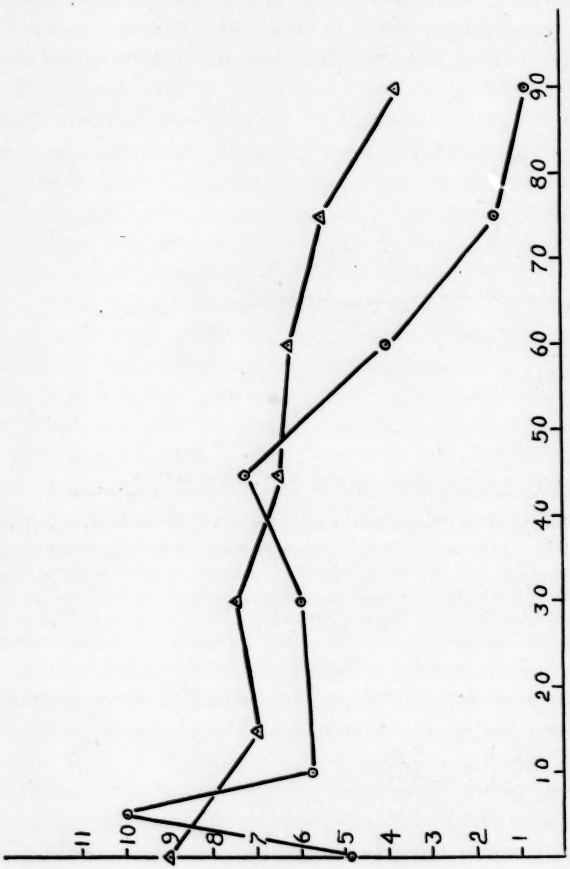

FIG. 15.—Graph showing changes in the volumetric ratio between plasmagel and plasmasol in two amoebae immersed in 0.15 M urea made up in $\frac{1}{10}$ modified Ringer solution. Abscissas, time from immersion in minutes; ordinates, plasmagel-plasmasol ratio. Note the gradual decrease in ratio after about fifty minutes, also the rapid initial increase in ratio and recovery in the first ten minutes in one amoeba; compare with volume changes in the same solution shown in Figure 12.

simultaneous with a rapid loss of total volume, and that as the rate of this loss decreases, the gel-sol ratio returns toward its initial value. Now it has also been shown that in urea the rate of loss in total vol-

ume, as in lactose, is first fairly rapid, but later decreases. It seems probable, therefore, that the gel-sol ratio also follows the same course as in lactose, i.e., that it follows the course indicated in the curve obtained by method B.

It is consequently concluded that, in all probability, the actual course of the changes in total volume and in gel-sol ratio, in the urea solution, is as follows: During the first fifteen minutes or so there is a decrease in volume accompanied by an increase in the gel-sol ratio, this is followed by a recovery, partial as to total volume, and complete as to the gel-sol ratio. Then, there is a short period of slight decrease in both the total volume and the gel-sol ratio, this decrease becomes continuously accelerated until, as previously stated, death results.

If this be true, on immersion of *Amoeba* in urea solution, water is first lost from the entire organism, but more rapidly from the plasmasol than from the plasmagel, then water re-enters the amoeba for a short time, after which this swelling gives place to a further loss of water, but this time water is lost more rapidly from the plasmagel than from the plasmasol, the rate of loss accelerating until death results.

The gradually accelerated parallel decrease in the gel-sol ratio and the total volume that occurred in the later part of the experiments in which urea was used (Figs. 12 and 15) indicates strongly that changes in permeability are correlated with changes in the extent of the plasmagel. This matter will be more fully considered presently.

Hydrogen-ion concentration.—Twelve amoebae were prepared as in the experiments on the relation between hydrogen-ion concentration and total volume, and in these the changes in the gel-sol ratio were measured by method B. The results obtained are presented in Figure 16.

From this figure it will be seen that there is a very definite relation between the hydrogen-ion concentration and the gel-sol ratio, that after three hours the gel-sol ratios found at pH 6.0, 6.6, 7.0, and 8.0 were 5.25, 4.25, 1.3, and 1.37, respectively, proving that there is a decided increase in this ratio, on the acid side, and a slight increase on the alkaline side. The fact that there was a slight increase

between pH 7.0 and 8.0 indicates that continuation of the experiments in the alkaline range would show an increase in the gel-sol ratio in this range similar to that found on the acid side.

Comparison of the results obtained in this experiment with those obtained in respect to the relation between hydrogen-ion concentration and total volume, presented in the preceding section (Fig. 13) demonstrates that there is a close relation, i.e., the gel-sol ratio and

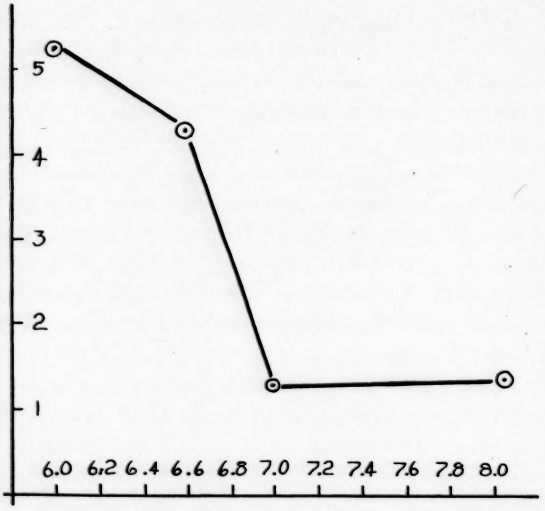

FIG. 16.—Graph showing the relation between plasmagel-plasmasol ratio and hydrogen-ion concentration. Abscissas, pH; ordinates, plasmagel-plasmasol ratio. Compare with Figure 13, showing volume changes in the same solutions.

the total volume increase or decrease *pari passu* as the hydrogen-ion concentration is altered. Thus, there is a correlation here between the thickness of the plasmagel and the water content of the cell, the two varying directly.

Mechanical stimulation.—The conclusions reached concerning the effect of mechanical stimulation on *Amoeba* are based upon observations with the volumescope on about one hundred amoebae. The results may be described as follows:

The direction of locomotion in *Amoeba*, while in the volumescope,

can be reversed by increasing the hydrostatic pressure at its anterior end. If the hydrostatic pressure at the anterior end of an amoeba moving in the capillary portion of the volumescope is increased, locomotion ceases at once, then there is a radial fissuring of the plasmagel at the posterior end. Through these fissures the plasmasol flows to form numerous fine pseudopodia which coalesce about the remaining masses of the old plasmagel. After this occurs the old gel rapidly disintegrates and a new anterior end is formed from the plasmagel of the coalesced pseudopodia. This then bulges out and locomotion begins in the new direction. On the contrary, if the hydrostatic pressure is increased at the posterior end, it simply results in a more rapid bulging of the anterior end and an increased rate of locomotion.

If reversal of the direction of locomotion has been induced as described in the foregoing, and an attempt is immediately made to again reverse the direction of travel, no fissuring occurs at the newly formed posterior end. There is simply a thinning of the plasmagel under the pressure, and a reversal of streaming, the newly formed plasmagel reverting to the sol state over a large part of the surface free in the tube, and then as the exterior membrane (plasmalemma) bulges forward locomotion begins. However, if a few minutes intervene before a second reversal is attempted, the fissuring and the other associated processes occur as described.

These facts indicate that there is a decided difference in rigidity between newly formed and old plasmagel, and that this difference is related to the age of the gel, the old gel being more rigid than the new. This tends strongly to support the contention of Mast (1926) that such a difference exists.

In these observations it was also noted that if the reversal is repeated at frequent intervals, i.e., approximately once a minute, for twenty or thirty minutes, reconstruction of the plasmagel becomes progressively more and more sluggish and its dissolution at each reversal more extensive. In fact, it gradually comes to involve the entire plasmagel, locomotion ceases as a normal process, and the entire mass within the plasmalemma becomes plasmasol. From this it is concluded that mechanical stimulation decreases the gel-sol ratio. Taking this fact into consideration together with the fact, as

demonstrated in the previous section that mechanical stimulation causes a loss in volume which increases as the stimulation is increased, it being easy to cause a loss of 12 per cent, it is further concluded that breaking down of the plasmagel renders *Amoeba* more permeable, and results in a decrease in water content and in volume.

DISCUSSION

In the preceding pages, it has been demonstrated that *Amoeba*, on immersion in a hypertonic solution of lactose or urea, decreases in volume, but that in lactose the rate of decrease decreases with time, while in urea it increases. It has also been demonstrated that in lactose solution the gel-sol ratio at first increases slightly and then returns toward its initial value after which it remains practically constant, while in the urea solution this ratio, after undergoing a similar change, does not remain constant but continues to decrease.

These facts indicate that in both solutions water is abstracted from the amoeba, first more rapidly from the plasmasol than from the plasmagel and then vice versa; but that in lactose the decrease in water content of the entire organism and of the plasmasol tends to cease, while in urea it is accelerated. This leads to the conclusion that some factor becomes active in abstraction in urea that does not appear in lactose.

Evidence has been presented indicating that if *Amoeba* is transferred from a slightly acid culture medium to $\frac{1}{60}$ modified Ringer solution at different hydrogen-ion concentrations, it increases in volume in solutions that are more acid than the culture, and decreases in those that are more alkaline, and that the greatest decrease occurs in the neutral solution. It has been demonstrated that under these conditions the gel-sol ratio is lowest at neutrality and higher in either direction.

This indicates that the water content changes *pari passu* with the changes in the gel-sol ratio as it does in the latter part of the experiments in urea.

Finally it has been observed that when *Amoeba* is subjected to mechanical stimulation it decreases in volume, and that likewise the gel-sol ratio decreases.

It seems evident from the foregoing that the water content in

Amoeba varies sometimes directly and sometimes inversely with the gel-sol ratio. The question arises as to how this difference can be explained.

To attempt an explanation it is necessary first to examine the experimental results to see if they indicate the mechanism involved. It will be noted that the water content varies directly with the gel-sol ratio when *Amoeba* is subjected to different hydrogen-ion concentrations, and that the variation in both water content and in the ratio yields curves (Figs. 13 and 16) that are similar to the curves obtained for the changes in water content of an amphoteric hydrogel, such as gelatin gel subjected to like conditions. The changes in water content that occur upon mechanical stimulation, taken in connection with the accompanying changes in the gel-sol ratio, appear to indicate that the plasmagel serves to a large extent in retaining the water content of the amoeba, since its destruction by stimulation is followed by reduction in volume, i.e., in water content. Further, the initial portions of the curves obtained in urea and lactose, since they indicate that water is lost in hypertonic solutions more rapidly from the plasmasol than from the plasmagel, appear to indicate that in such retention of water the plasmagel acts as a semipermeable membrane with respect to the plasmasol. Finally the change in the curvature in the latter part of the curve showing the results in urea indicates that urea has upon the plasmagel a dispersive effect possibly similar to its effect upon gelatin gels (Bechold, 1905, p. 55).

Taking all these facts, and the fact that the activity of the plasmagel appears to be minimum at neutrality, as shown in Figs. 13 and 15, it appears reasonable to assume that the permeability in *Amoeba* and therefore its water content is to a large extent controlled by the plasmagel, and that the factor exercising this control is an amphoteric colloid, probably a hydrogel, within the plasmagel, which has an isoelectric point at about pH 7.0 and is affected by urea like gelatin.

On the basis of this assumption the following will obtain:

Amoeba immersed in lactose will lose in volume, owing to the osmotic pressure of the medium. However, the plasmagel will lose water less readily than the other cell constituents, since the colloid

in the plasmagel being hydrated (i.e., holding water by adsorption on its micellae, and hence not free to move as liquid) cannot part readily with its water content. As equilibrium is reached equalization of the normal relation will be restored, or nearly so provided that the loss of water of hydration from the colloid is slower than the loss of water from the cell due to osmotic pressure.

Amoeba immersed in hypertonic urea solution will at first be affected by the osmotic pressure just as in lactose, but due to the effect of the urea, the colloid after a short time will be broken down. Then the cell will become more permeable and this will result in a simultaneous decrease in the gel-sol ratio and in the volume, for the gel-sol ratio is controlled by the condition of the colloid, and as this is dispersed the ratio must change, and likewise since the condition of the colloid controls the permeability its dispersion will entail an increase in permeability with loss of salts and water content.

Amoeba immersed in hydrogen-ion concentrations more acid or more basic than the culture fluid will alter in volume just as does a gelatin gel under similar conditions, and like such a gel will have least volume at its isoelectric point. The gel-sol ratio, since it is dependent upon the hydration condition of the protein here assumed, will likewise alter, in fact the changes in volume described in the preceding are the changes in volume undergone by the plasmagel as distinguished from the plasmasol. That is, the changes in gel-sol ratio and volume will be due to increase or decrease of the hydration of the colloid at the different hydrogen-ion concentrations, with their consequent effects.

Mechanical stimulation will result in disintegration of the hydrogel and consequent loss of water content, since it is assumed that integrity of this gel is in large part responsible for the semipermeability of the cell.

In regard to such injury by mechanical stimulus, it might be suggestive to consider the possibility of the colloid being present within the plasmagel in the form of a film structure such as described by Mast (1926*a*), i.e., as filling the interstices between the included vacuoles and granules. If this obtains mechanical stimulation might cause rupture of the films, thus lessening the thickness of the plasmagel as a whole. Also it is pertinent to note that Lepeschkin (1926) asserts that mechanical stimulation of proto-

plasmic colloids results in coagulation, and that Gurchot (1926) contends that partial coagulation of membranes will render them more permeable. Hence, applied to the plasmagel, admittedly colloid in structure, the findings of these two investigators would lead to the expectation that mechanical stimulation would lead to an increase of permeability in any cell membrane, with consequent loss in volume.

The question now arises as to what bearing the demonstrated facts, taken in connection with the hypotheses advanced in explanation of them, have upon the problem of the gel-sol transformation in locomotion.

As stated in the foregoing the colloid of the plasmagel was assumed to be of importance in respect to the permeability of *Amoeba*, and it was also implicitly assumed that its thickness is a measure of its effectiveness. It has been definitely shown, except under conditions in which osmotic pressure is presumably the sole agent, that when a change in volume occurs in *Amoeba* which requires for its explanation the postulation of changes in permeability, the water content changes directly with the gel-sol ratio. This indicates, if the postulated colloid exists, that hydration of a protein or similar substance is a factor in the gel-sol transformation, for it is obvious that this colloid must be present both in the plasmagel and the plasmasol, and increase of the plasmagel relative to the plasmasol, with increasing water content, must mean an increase in extension of the hydrated colloid in the plasmagel with increase of hydration of the cell, and a decrease of this with dehydration. These experiments therefore are in line with the conclusions of Pantin (1925) that a process of hydration and dehydration occurs as an integral part of the process of locomotion, since it would appear from the foregoing that it must be considered that the plasmagel is formed from the plasmasol by imbibition of water, or absorption, of it, on the particles of the colloid.

The findings also support Mast's contention (1926*b*) that the plasmagel plays the part of a semipermeable membrane with respect to the plasmasol.[1]

[1] The writer desires to express his sincere thanks to Professor S. O. Mast, who suggested the problem and under whose guidance the work was done, for his continued

SUMMARY

1. Two methods are given for the measurement of volume in *Amoeba*. In one the volume is ascertained by calculation from measurements on outline drawings of *Amoeba* as seen from above and from the side. In the other it is ascertained by measurement after the amoeba has been caused to assume the form of a cylinder of known diameter. The methods are accurate to somewhat less than 10 and 5 per cent, respectively.

2. *Amoeba* immersed in hypertonic solutions of glycerol, lactose, or urea decreases in volume. In glycerol solutions the volume varies directly with concentration, over the range employed. In lactose and glycerol the rate of decrease in volume diminishes with time until the volume becomes constant. *Amoeba* transferred from $\frac{1}{30}$ modified Ringer solution at pH 6.8 to 0.15 M lactose solution of the same C_H loses approximately 50 per cent in volume. In urea the rate of decrease in volume first diminishes and then increases with time until death. In glycerol and lactose the decrease in volume is probably the result of loss of water by diffusion, owing to the hypertonicity of the medium. In urea it is probable that while this obtains at first there is later a loss of water owing to increase in permeability brought about by the action of the urea on the protoplasm.

3. Mechanical stimulation breaks down the plasmagel structure in *Amoeba*, and this is accompanied by a decrease in water content. High frequency stimulation is more effective than low.

4. *Amoeba* transferred from a culture at pH 6.8 to a series of $\frac{1}{30}$ modified Ringer solutions of different hydrogen-ion concentrations loses water most rapidly at neutrality, and gradually less rapidly in either direction. On the acid side the loss is replaced by a gain in solutions more acid than pH 6.4. It is probable that *Amoeba* if transferred from a slightly acid culture medium to a more acid medium will gain in volume, and that if it is transferred to more alkaline media it will lose.

5. In hypertonic solutions of urea the volumetric ratio between the plasmagel and the plasmasol decreases after a sharp initial increase. This decrease is accelerated with time. The initial change

interest and encouragement, and for assistance and criticism in preparation of the manuscript for publication.

is probably due to a disturbance of equilibrium across the plasmagel. The final decrease is probably due to the action of the urea on a colloid structure in the plasmagel, similar to its action upon gelatin gels, leading to an increase in the permeability of the cell which results in entrance of urea and either destruction of the colloid structure or interference with the plasmagel-plasmasol transformation.

6. In hypertonic solutions of lactose the volumetric ratio between plasmagel and plasmasol at first increases and then returns toward its initial value. The initial change is probably due to disturbance of equilibrium across the plasmagel which probably holds part of the water contained in a bound condition, and therefore although in fact more hydrated than, or at least equally hydrated with, the plasmasol, acts as if less hydrated, the plasmasol water being free, being more readily abstracted from the cell. The recovery later is due to transformation of plasmagel to plasmasol or redistribution of water content within the cell, which restores the equilibrium between free and bound water (i.e., water adsorbed and forming an integral part of the colloid structure of the plasmagel).

7. The volumetric ratio between plasmagel and plasmasol is dependent upon the hydrogen-ion concentration of the surrounding medium. The ratio varies from 5.25 at pH 6.6 to 1.4 at pH 7.0 and 8.0. There is probably a tendency to an increase in this ratio in alkaline solutions as compared with neutral solutions.

8. The transformation of plasmasol to plasmagel in *Amoeba* is apparently the result of the formation within the plasmasol of a colloid interprotoplasmic structure, and the transformation of plasmagel to plasmasol the result of the dissolution of this structure. It is postulated that the formation of this structure is the result of hydration of micellae composed of an amphoteric substance with an iso-electric point at about pH 7.0, and its dissolution is due to their dehydration, which results in flocculation.

9. Since increase and decrease in water content vary directly with the thickness of the plasmagel, except under conditions in which osmotic pressure is probably the sole agent effecting the transfer of water through he cell surface, it appears that in *Amoeba* changes in permeability are probably functions of the plasmagel.

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STUDIES ON AMPHIBIAN METAMORPHOSIS. V. THE ATROPHY OF ANURAN TAIL MUSCLE DURING METAMORPHOSIS

(Five figures)

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THE total atrophy of the larval tail constitutes one of the most striking changes evidenced during anuran metamorphosis. The sequence of gross degenerations is quite definite although the time relationships between tail atrophy and other metamorphic changes is subject to some variation. As regards the former point, it may be said that the dorsal and ventral finny portions of the integument first degenerate, followed shortly by the musculature beneath. Evidence is also presented in the present article to support the idea that the anterior portions of the tail undergo atrophy more rapidly than more posterior regions. In natural metamorphosis the structure as a whole is apparently unaffected during early stages of larval transformation, suggesting the idea that the secretions of the thyroid are not directly concerned in initiating the degeneration. It is also a peculiar fact that in artificial metamorphosis the atrophy of the tail occurs much earlier in relation to other signs of transformation than is normally the case.

The problems concerned in tail atrophy may be classified under three main headings, viz., cytological, involving histolytic cellular changes; biochemical, embracing early chemical changes and later progressive changes during the ensuing histolysis; and causative problems as to the immediate fundamental agents initiating the various biochemical and cytological reactions which result in atrophy. Since the cytological and biochemical findings have been adequately reviewed elsewhere (Morse, 1918; Bradley, 1922) only the causative phase will be taken up here.

In 1891 Bataillon found that the development of the urostyle brought about a redistribution of the vascular supply to the tail owing to pressure of the rapidly growing organ on blood vessels

entering the tail. Mercier (1906) identified phagocytes in the atrophying musculature of the tail which contained carmine granules previously injected into the dorsal lymph sac, thereby showing that complete occlusion of the vascular supply was not involved. Both of these workers suggested the probability that the reduced blood supply to the tail was responsible for the accumulation of metabolites with resultant tissue acidosis and autolysis. Morse (1918), commenting on this explanation of anuran tail atrophy, thought that a generalized probable lowering of the blood hydrogen-ion concentration below pH 7.0 was a more logical explanation although no blood determinations were made to lend evidence to this conception.

More recently Helff (1928), in a preliminary article, has shown that the urostyle cannot be the main causative factor since normal atrophy occurs following the removal of the larval urostyle before the latter undergoes growth. Simultaneously with this finding, Lindeman (1929) was able to demonstrate the degeneration of tail integument during metamorphosis when previously transplanted to the backs of tadpoles. These two results would tend to support the idea that the tissues of the larval tail are peculiarly susceptible to influences operative through the blood supply at a certain stage of metamorphosis, although whether these influences consist of hormonic, acid, or other changes is quite problematic. The present article describes the results of tail and other muscle transplantations designed to throw light on the probable causative factors responsible for autolysis of the musculature of the tail as it occurs during larval transformation.

MATERIALS AND METHODS

The stock used for all operations were large *Rana clamitans* larvae obtained from the vicinity of Cincinnati, Ohio, during the months of January, February, and March, 1928. These partially neotenous forms were in their second year's growth and averaged about 80 mm. in total length. Individuals were selected which were normal larvae in all respects, the only signs of transformation being the presence of small 3 to 5 mm. hind limbs. Such individuals remain unchanged in the laboratory as typical larvae during the winter and spring months and it was therefore necessary to feed

with desiccated thyroid in order to induce metamorphosis essential for the purposes of the experiments.

The technique employed in making muscle transplantations was briefly as follows: A flap of integument was first laid back and a small piece of muscle removed and placed in a few drops of lymph in a watch crystal. This piece of muscle was then accurately shaped under a dissecting microscope so that a 2-mm. cube of tissue was prepared for transplantation. In transplanting, a flap of integument was likewise laid back from the region to receive the transplant, the underlying musculature lightly scraped, and the cubical muscle transplant placed on this surface. In some cases the transplant was partially inserted in a small incision made in the muscle. The integument in either case was then replaced and the animal placed in shallow water to expose the operative area to the air and so hasten the adherence of the skin. They were then finally submerged in water to recover from the effects of the chlorotonization. All operations were autoplastic and in many cases were reciprocal, i.e., from back to tail and from tail to back of the same individual.

Following recovery from the anesthetic, the operated individuals were placed in individual containers and maintained under constant laboratory conditions. At the end of three days the integument covering the transplant was laid back and the latter examined under a dissecting microscope and generally found to be firmly attached and healthy in all respects. All cases of incomplete or abnormal grafting were discarded. The flap of integument was then replaced and the individuals fed desiccated thyroid to induce metamorphosis. The transplants were likewise examined at ten-, fifteen-, and twenty-one-day intervals following the application of the desiccated thyroid, during which time the larvae were undergoing transformation. Each time an examination was made the transplant was carefully measured, thus making possible a fairly accurate determination of its volume. In this way, knowing the original volume of 8 cu. mm., it was possible to determine the reduction in volume due to atrophy quite accurately. Typical examples of transplants examined at three-, ten-, fifteen-, and twenty-one-day intervals were selected from all operative series for sectioning and study of the histological condition of the muscle grafts.

RESULTS

1. *Transplantation of tail muscle to the back.*—The purpose involved in making tail-muscle transplants to the back of larvae was primarily to see whether or not such tissue would undergo normal atrophy at the same time the musculature of the tail degenerates. Failure of such transplants to histolyze would indicate that local influences peculiar to the tail region are normally operative while the reverse result would suggest a more generalized influence applied by way of the blood stream.

The muscle tissue transplanted was obtained from the side of the tail at a level about equidistant from the tip of the organ and its

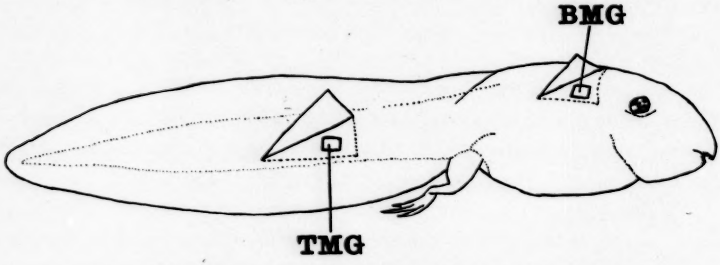


FIG. 1.—Diagrammatic outline illustrating operative procedure

junction with the body (*TMG*, Fig. 1.). The muscle, after being shaped as previously described, was then transplanted, autoplastically, to a position a little to one side of the middorsal line on the back (*BMG*, Fig. 1). This insured adequate contact of the graft with the muscle of the back; transplantation to a middorsal location being inadvisable owing to the thinness of the musculature of that region.

One hundred and thirty successful transplantations were made of this type, the percentage of abnormal grafting being very small —about 1 per cent. When examined three days following the operation and just prior to the desiccated thyroid feeding, the transplants on the average gave evidences of a slight decrease in volume. This initial shrinkage, although comparatively slight (8–11 per cent), seems to be characteristic of muscle transplantation in frog larvae and is probably the result of healing and other adjustment factors.

The reduction is not due to histological degenerative changes as can be shown by sectioning. In either case the amount of reduction was too slight to effect seriously the decided histolytic reduction in transplant volume experienced during metamorphosis.

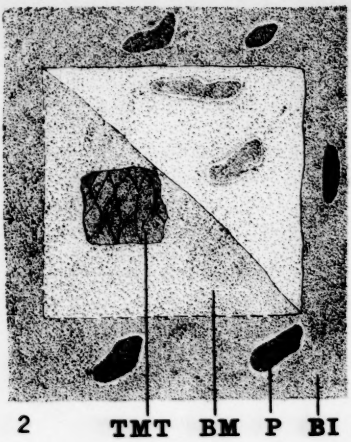
Table I represents the essential volume-reduction data, due to histolysis, for the four types of transplantations made. It will be noted that the reduction in volume of tail-muscle transplants to the back proceeds quite steadily during the period of artificially induced metamorphosis. At the end of the first ten days, the volume of tissue remaining is slightly less than half. The reduction in volume

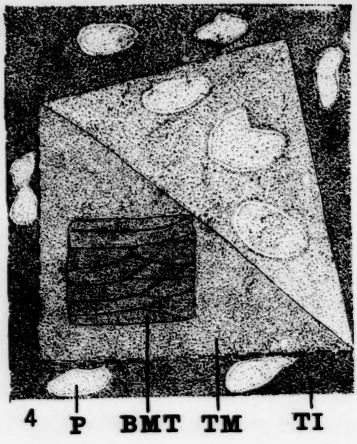
TABLE I

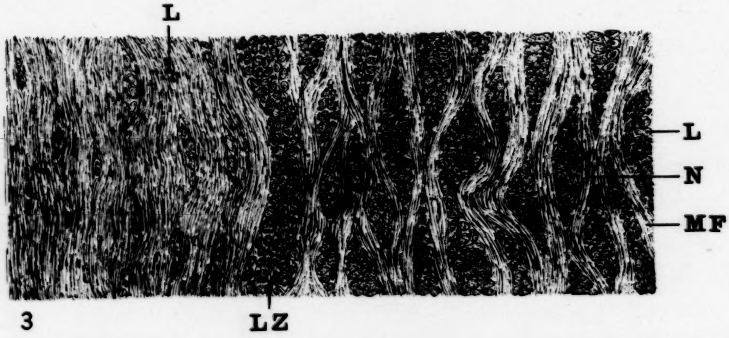
Type of Transplantation	Number of Cases	Average Reduction in Volume Due to Atrophy		
		10 days	15 days	21 days
Tail muscle to the back	130	58%	86%	100 cases—100% 30 cases— 99.2%
Back muscle to the tail	60	21%	48%	54%
Tail muscle to other side of tail	15	38%	40%	49%
Back muscle to other side of back	15	27%	38%	38%

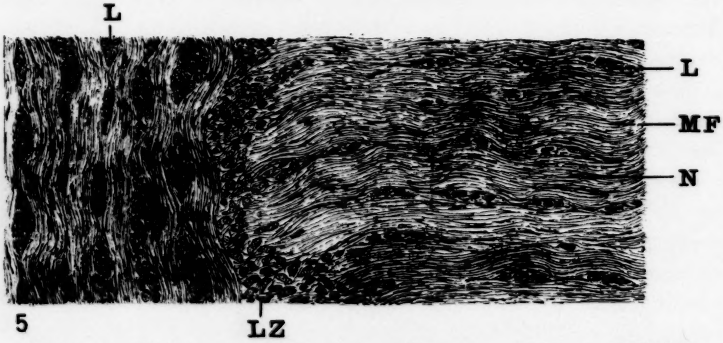
continues at a uniform rate and in the majority of cases results in the total obliteration of the graft by the end of twenty-one days. There is a small amount of variation between different individuals regarding the rate of atrophy, but in no case could more than 3 per cent of the original volume be detected at twenty-one days. The small remnants of tissue persisting in thirty cases as indicated in Table I were found to disappear within a few days.

Figure 2 represents the typical appearance of a tail-muscle graft on the back at the fifteen-day examination period. In linear dimensions the graft has been reduced from a 2-mm. cube to a 1.05-mm. cube, or a reduction in volume of 85.5 per cent. In external appearance the graft appears fairly normal, the bundles of muscle strands being still well indicated although the tissue as a whole appears slightly grayish in color as compared with the whiter shade of freshly transplanted muscle. Figure 3 represents a histological section taken through part of such a transplant to include a portion of the adjoining back muscle. It will be noted that the muscle bundles









FIGS. 2–5—*BI*, integument of the back; *BM*, muscle of the back; *BMT*, back-muscle transplant to the tail; *L*, phagocytic cells, chiefly lymphocytes; *LZ*, zone of phagocytic cells between transplant and host muscle; *MF*, dissociating muscle bundles; *N*, nuclei; *P*, pigment spots; *TI*, integument of the tail; *TM*, muscle of the tail; *TMT*, tail-muscle transplant to the back.

FIG. 2.—Semidiagrammatic sketch of a tail-muscle transplant to the back fifteen days following the onset of metamorphosis. The transplant has atrophied approximately 86 per cent in volume.

FIG. 3.—Section through tail-muscle transplant and adjacent back muscle as shown in Figure 2. Tail-muscle transplant shown to the right of *LZ;* surrounding back muscle pictured to the left of *LZ*.

FIG. 4.—Semidiagrammatic sketch of a back-muscle transplant to the tail fifteen days following the onset of metamorphosis. The transplant has atrophied approximately 48 per cent in volume.

FIG. 5.—Section through back-muscle transplant and adjacent tail muscle as shown in Figure 4. Back-muscle transplant shown to the right of *LZ;* surrounding tail muscle pictured to the left of *LZ*.

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of the graft are rapidly undergoing dissociation while the tissue is heavily invaded by lymphocytes and other phagocytic cells typical of normal muscular autolysis of the tail. Contrasted to this the adjoining back muscle is much less affected, although slight signs of dissociation can be detected. Usually a heavy discontinuous zone of phagocytic cells is to be found between the transplant and host tissue. This does not necessarily indicate that the peripheral regions of the transplant always undergo more rapid histolysis than more central regions since junction points between graft and host tissue are not uncommonly found containing few, if any, phagocytes.

2. *Transplantations of back muscle to the tail.*—The reverse transplantation of back muscle to the tail was thought to be of interest in that a possible resistance of back muscle to histolysis might be shown to exist, although the fact that it would be surrounded, during metamorphosis, by rapidly autolyzing tail muscle might well serve to mask such a resistance.

The muscle transplants were obtained from regions to one side of the back (*BMG*, Fig. 1) and transplanted to the sides of the tail (*TMG*, Fig.1). Sixty successful transplantations were obtained in all that appeared healthy and firmly attached three days after the operation. The average behavior during metamorphosis as regards volume changes is indicated in Table I. Reduction in size invariably occurred although with more variation between individuals than was true of the tail-muscle grafts as previously described. Figure 4 represents the macroscopic appearance of a typical graft examined fifteen days after the initial desiccated thyroid treatment. The grafts at this time had been reduced in linear dimensions from a 2-mm. to a 1.6-mm. cube, or a volume reduction of approximately 48 per cent. Sections made through such transplants (Fig. 5) show that atrophy is brought about through the usual methods of autolysis, the muscle fibrils being dissociated although not to as great an extent as is true of the adjacent atrophying tail muscle. Phagocytic invasion was also less pronounced in such transplants as compared with the atrophying tail musculature.

3. *Transplantation of back and tail muscle to adjacent regions.*—Histological sections through tail-muscle transplants on the back and the adjacent musculature of the back gave indications that the

latter tissue is evidently normally subjected to autolytic reactions during metamorphosis. If this were true, the rate of atrophy of foreign transplants to the back might well be increased above their normal speed. It was thought advisable, therefore, to determine the normal rate of back-muscle atrophy during larval transformation.

The operation involved consisted of the transplantation of an 8-cu. mm. piece of back muscle from one side to the other of the middorsal line on the same individual. The animals were then artificially metamorphosed and periodic examinations made of the transplants in the usual manner. It was at once evident that the back muscle of involuting larvae is subject to a decided atrophy. Reference to Table I will show that within fifteen days, following feeding of desiccated thyroid, the volume of the transplants had been reduced approximately 38 per cent. Histological examination of the transplants showed that the same type of dissociation was present as previously noted in back-muscle transplants grafted to the tail. It was furthermore noted that practically no further atrophy occurred during the next six days, at which time the transplants were again examined and measured. It would seem evident, therefore, that the musculature of the back undergoes a certain degree of atrophy, the end-stage of which is reached some time before the close of metamorphosis.

It was likewise thought profitable to determine the approximate rate of tail-muscle atrophy *in situ*. The transplantation in this case consisted of removing muscle from one side of the tail and grafting it to the opposite side of the same organ. During larval transformation the behavior of such transplants could be determined and data obtained regarding the normal rate of tail-muscle atrophy.

Table I gives the average percentage reduction of fifteen such transplantations at three stages of metamorphosis. It is evident that the rate of autolysis resulting in atrophy is quite uniform and of course progressive. The degree of atrophy also serves to illustrate the stage of metamorphosis at which the three examination periods for the other types of transplantations were made. Thus the ten-day examination was made when the volume of the tail had been reduced 27 per cent, the fifteen-day examination when the atrophy

was 38 per cent, and the twenty-one-day examination when approximately 50 per cent, or one-half, of the tail had been resorbed.

It was consistently observed that muscle transplants to the tail invariably tended to move toward the junction of body and tail as metamorphosis and the resorption of the tail progressed. This did not indicate that there was an actual migration of the graft in any sense but rather that the anterior portions of the tail were undergoing a more rapid rate of atrophy than more posterior portions. If, for example, the transplant had originally been placed at a point exactly halfway distant from the anterior and posterior limits of the tail, examination twenty-one days following the onset of metamorphosis would show that it was now fairly close to the junction point of body and tail while the original distance between transplant and the tip of the tail had not been greatly diminished. The resorption of the tail would therefore appear to be more rapid in the more anterior regions, which fact probably accounts for the well-known attenuated appearance of atrophying tails.

In a histological study of the autolyzing tail musculature of involuting *Rana pipiens* larvae, Morse (1918) describes and pictures (p. 152) "dark masses running transversely over the muscle bands," which he defines as "depositions of fats bearing unsaturated fatty acids (oleic) which stain with osmium tetroxide." This condition, he further states, is precursory to phagocytosis. During the course of the present work a variety of osmium tetroxide staining methods have been used on normal tail muscle and muscle in different stages of atrophy. Our slides show only occasional depositions as described by Morse, and although their occurrence when present may always precede phagocytic invasion as he has stated, it is also true that the great bulk of tail muscle at the onset of autolysis and just prior to phagocytic invasion presents no striking reactions to osmic acid.

DISCUSSION

Consideration of the relative degree of atrophy of the various muscle transplants during metamorphosis as compared with the normal atrophy of the same tissue *in situ* during the same time interval calls for some explanation. Thus the musculature of the tail reduces 49 per cent within twenty-one days while the same muscle

transplanted to the back undergoes 100 per cent or complete resorption within the same period of time. Part of this increased rate of histolysis can be explained by the 8–10 per cent reduction in volume to which muscle tissue appears to be subject upon transplantation. The remaining further approximate increase of 41 per cent might possibly be explained by assuming that the normal 38 per cent reduction of the surrounding back muscle is additive in effect on the rate of atrophy of the tail-muscle transplants. Similarly, the 38 per cent normal reduction of back muscle, which increases to approximately 54 per cent within twenty-one days when transplanted to the tail, may be due to the usual 10 per cent transplantation reduction and to the influence of the rapidly degenerating surrounding musculature of the tail.

The suggestion might well be advanced that a comparatively highly differentiated tissue-like muscle would be likely to undergo complete resorption when transplanted for some time in non-metamorphosing larvae. In order to determine whether or not this is true of the different types of muscle transplanted in the present work, the following tests were made: ten transplantations each were made of tail muscle to the back, back muscle to the tail, back muscle to another region on the back, and tail muscle to the opposite side of the same tail. All transplantations were autoplastic and made on normal larvae showing no signs of transformation. The operated animals were maintained in cold water and gave no evidence of metamorphic changes within twenty-one days, at which time the transplants were examined. The average reduction in volume of the various muscle transplants was as follows: tail muscle to the back, 10 per cent; back muscle to the tail, 9 per cent; back muscle to another region on the back, 11 per cent; and tail muscle to the opposite side of the same tail, 8 per cent. It would appear clear, therefore, that the normal reduction in volume due to transplantation of the foregoing types of muscle could not have accounted for the much greater histolytic atrophies recorded during metamorphosis.

The results of the present article indicate, therefore, that the atrophy of anuran tail muscle during metamorphosis is not due to autolytic factors exclusively peculiar to the tail. The complete re-

sorption of tail muscle during metamorphosis when transplanted to the back would rather suggest that the causative factors of atrophy are generalized and transported through the blood stream. The nature of this histolytic influence is, of course, quite problematic. Morse (1918) suggests that partial interruption of the blood supply everywhere results in the accumulation of carbon dioxide to the extent that the "buffer" mechanism of the blood is soon neutralized, resulting in an actual acidity of the blood. If this were true, the explanation would be sufficient to account not only for tail-muscle autolysis but for that of all other muscle undergoing similar degeneration during involution. The authors of the present article are favorably inclined toward this idea in that preliminary hydrogen-ion determinations of involuting larvae lymph indicate an increase in acidity. Work is also under way to determine the hydrogen-ion changes of anuran blood during metamorphosis. There is also a possibility that the thyroid hormone may be directly concerned. It is doubtful, however, that this is the case since the musculature of the tail remains normal for some time following the onset of metamorphosis and at a time when many other parts and organs are undergoing degeneration, development, or dedifferentiation due to the direct or indirect influence of the thyroid hormone.

SUMMARY AND CONCLUSIONS

1. Reciprocal autoplastic transplantations of *Rana clamitans* tail and back muscle were made to determine the degree of atrophy occurring during larval transformation. The normal atrophy changes of the musculature of the back and tail were likewise investigated by transplantation to adjacent regions.

2. The musculature of the tail and back *in situ* was found to atrophy approximately 49 and 38 per cent, respectively, within twenty-one days following the onset of metamorphosis. During the same time interval, back muscle previously transplanted to the tail was 54 per cent resorbed while tail muscle previously transplanted to the back was completely obliterated.

3. Control transplantations of various muscle tissues maintained on non-metamorphosing larvae for twenty-one days gave no evidence of a sufficient amount of atrophy to affect seriously the results

of muscle-transplant atrophy as determined during metamorphosis.

4. The authors conclude that the atrophy of anuran tail muscle during involution is not due to histolytić influences exclusively peculiar to the tail but rather to more generalized factors transported by the blood stream, the probable development of an actual acidity of the blood being perhaps the main causative factor.

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